CARBOHYDRATE NUTRITION IN SOME SELECTED CULTIVABLE FINFISH SPECIES

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF

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ABSTRACT

Compared to protein and fat, carbohydrates are cheap energy yielding nutrients in many fish diets Similar to fat, adequate levels of carbohydrate inclusion in fish diets spare costly dietary protein for growth purposes, leading to cost-effective and successful feeding strategy for semi-intensive/intensive fish culture Although omnivorous carps efficiently make use of dietary carbohydrates for energy purposes, disparity exists in the carbohydrate utilization by these fishes The present study was undertaken to evaluate dietary carbohydrate utilization in the three species of the Indian major carps, and two species of commercially important catfish, and results are compiled in the form of the present thesis

All experiments were carried out using completely randomized design Each dietary treatment had three replicates Fishes were stocked in 70 l high density polyvinyl flow-through (1-1.5 l/min) indoor circular troughs (water volume 55 l) and fed to apparent satiation, six days a week, twice daily at 0800 and 1600 hours Fish were bulk weighed on weekly basis The effectiveness of experimental diets were assessed in terms of growth, conversion efficiency, body composition and nutrient retention

Effects of varying levels (0,5,10,15,20,25 or 30%) of dietary carbohydrate have been evaluated on the growth, conversion efficiencies, body composition, and nutrient retention efficiency of young catfish, *H. fossilis* (10 80±1 7 cm, 8 15±0 23 g) Maximum growth, conversion efficiencies, and increased nutrient retention were seen in fish at 20% dietary carbohydrate and 3 23 kcla g^{-1} (ME), corresponding to an energy to protein ratio of 8 08 kcal g^{-1} protein Changes in the wholebody composition were significantly affected by the levels of carbohydrate intake Increase in dietary carbohydrate resulted in higher values for dry matter, fat and energy contents

Crude protein and ash remained unaffected *(P > 0.05)*, while moisture decreased *(P < 0.05)* with increase in dietary carbohydrate levels Protein and energy retention efficiencies increased linearly up to 20% carbohydrate inclusion in the diet beyond which a significant fall *(P < 0.05)* in the values appeared However, fat retention continued to increase progressively with carbohydrate intake A four-fold increase in liver and gut weights, and a two-fold increase in hepato- and gastro-somatic indices were observed at 30% dietary carbohydrate

Growth, conversion efficiency, body composition, nutrient retention and plasma glucose concentration were evaluated in fingerling catfish, *H. fossilis* (6 20±1 0 cm, 3 27±0 04 g), fed iso-nitrogenous (40% CP), iso-caloric (4 70 kcal g^{-1}, gross energy) test diets, containing different sources of carbohydrate (glucose, fructose, maltose, sucrose, dextrin, pre-cooked corn starch or α- cellulose), at 20% level of inclusion Maximum growth and feed conversion efficiencies (*P < 0.05*) were obtained with dextrin containing diet Growth and conversion efficiencies were minimum in fish fed α-cellulose based diet Variations in plasma glucose were significant (*P < 0.05*) with respect to level (maximum and minimum) and recovery over the 8 hour sampling time Post-feeding glucose or maltose resulted in maximum increase in plasma glucose, followed by sucrose, fructose or dextrin containing diets Post-feeding pre-cooked corn starch or α-cellulose produced relatively low (*P > 0.05*) variation in plasma glucose All experimental groups at the end of the study exhibited significantly (*P < 0.05*) higher percentages of crude lipid, ash and energy, and lower percentage of body moisture Maximum protein retention was noted with dextrin containing diet, while higher fat and energy retention was seen with sucrose based diet Dietary α-cellulose produced significantly lower (*P < 0.05*) values for protein, fat and energy retention

Effects of varying carbohydrate-to-lipid (CHO L) ratios (0 02, 0 60, 1 54, 3.38, 8.93 or 43.00 g:g) on the growth, conversion efficiencies, body composition, and nutrient retention efficiency were studied in the Indian major carps fry, *C. catla* (2.10±0.5 cm; 0.20±0.01 g), *L. rohita* (2.10±0.4 cm; 0.17±0.01 g), and *C. mrigala* (2.20±0.8 cm; 0.31 ±0.01 g), and in young walking catfish, *C. batrachus* (12.50±.2 cm; 13.04±0.11 g). Growth rates in fishes differed significantly *(P < 0.05)* with CHO:L ratio in the diets, producing a quadratic pattern. Maximum weight gain (%) and SGR(%) were observed in *C. mrigala* and *C. batrachus* with 8% lipid and 27% carbohydrate diet, corresponding to a CHO:L ratio of 3.38. *C. catla* and *L. rohita* showed maximum weight gain and SGR at 4% lipid and 36% carbohydrate, corresponding to CHO:L ratio of 8.93. In all the species, fish fed either the lowest (0.02) or the highest (43.00) CHO:L ratio tended to produce significantly lower *(P < 0.05)* growth and conversion efficiencies. At the end of the study, all experimental groups exhibited higher perecentages of crude protein, fat and body energy, and lower percentages of moisture and ash. Highest protein and energy retention efficiencies were noted in fish with maximum weight gain and body crude protein. Fat retention efficiency increased progressively with dietary CHO:L ratio. The finding suggests optimal carbohydrate and lipid levels in 40% CP and 3.46 kcal.g^{-1} ME diet to be 36% and 4%, respectively, corresponding to CHO:L ratio of 8.93, for the fish species investigated.

Influence of varying levels (8, 16, 24, 32, 40 or 48%) of dietary carbohydrate (bread flour) has been examined on the growth, conversion efficiency, body co mposition and nutrient retention efficiency of *C. catla* (3.0±0.5 cm; 0.28±0.02 g) fry. Weight gain (%) was significantly affected *(P < 0.05)* by carbohydrate intake, resulting in a quadratic growth pattern. Second degree polynomial regression analysis indicated that maximum SGR (3.41%) would occur at 37.69% carbodydrate level, with an E/P ratio of 9.03 kcal.g^{-1} protein and 3.61 kcal.g^{-1} (ME) diet The relationship of dietary carbohydrate (up to 40% inclusion) with SGR, PER and nutrient retention was linear and poisitive However, this relationship was negative with FCR Dietary carbohydrate significantly altered *($P < 0.05$)* carcass moisture, crude fat and gross energy, while crude protein and ash remained unaffected

Protein-sparing effect of different dietary carbohydrates was examined in fingerling *L. rohita* (4 10±0 7 cm, 2 03±0 01 g) fed three levels (40, 35, or 30%) of protein with three levels (30,35 or 40%) of different carbohydrate sources (glucose, sucrose or dextrin) Weight gains, FCR, PER and SGR remained unaffected *($P > 0.05$)* with increase in carbohydrate inclusion from 30-40% and a concomitant decrease in dietary protein level from 40-30%, when dextrin was used as a carbohydrate source These values were moderate with sucrose based diets However, poor values for the above were noticeable in fish fed glucose containing diets Marked difference was noticeable in the body composition of fish fed different test diets At each protein level, with greater complexity of carbodydrate, body dry matter, crude protein, total lipid and gross energy increased significantly *($P < 0.05$)*, while body ash content remained low showing no discernable change with dietary treatments Increase in the levels of sucrose and dextrin in the diet produced significantly higher *($P < 0.05$)* protein and energy retention values, while these values significantly decreased *($P < 0.05$)* with incremental dietrary glucose levels The study suggests that in *L. rohita,* dextrin, as a carbodydrate source, is better utilized and has a greater protein-sparing effect than sucrose or glucose

Evaluation of apparent dry matter, nutrient (crude protein, fat and carbohydrate) and energy digestibility coefficients for nine raw and two steam-cooked agro-based by-products in pelleted feed (70 30 reference test ingredient), containing 30-32 % CP, 3 9-4 3 kcal g^{-1} gross energy, and 1 % chromic oxide, was carried out in fingerling *C. catla* (4-6 cm, 1 5-2 5 g), *L. rohita* (3 9-6 2 cm, 1 5-2 6 g), and *C.*

mrigala (4.1-6.0 cm; 1.6-2.8 g). Within each test species, apparent dry matter, nutrient and energy digestibility coefficients varied significantly *($P < 0.05$)* with the feedstuffs tested. However, with few exceptions, digestibility coefficients for an individual test ingredient varied insignificantly *($P > 0.05$)* among the three fish species. The study indicates that feedstuffs rich in carbohydrate and energy contents are effectively utilized by the three species of the Indian major carps. The variations observed in dry matter, nutrient and energy digestibility coefficients in the three fish species seemed related to the source and nutrient composition of the feedstuffs. Feedstuffs and test diets with higher levels of fibre and ash showed significantly low *($P < 0.05$)* values for dry matter, nutrient and energy digestibility. Steam-cooking of corn and potato significantly *($P < 0.05$)* improved the digestibility coefficients in the test species. The findings of the present study are important in developing low-cost balanced rations, incorporating locally available agro-based by-products, for the polyculture of the three species of Indian major carps.

The data generated during the study provide basic information which could of interest to fish farmers and nutritionists in compounding cost-effective artificial rations for these fishes.

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Professor

Certified that the work entitled ***"CARBOHYDRATE NUTRITION IN SOME SELECTED CULTIVABLE FINFISH SPECIES"*** has been completed under my supervision by ***Mr. Erfanullah***. The work is original and has been independently pursued by the candidate. It reports some interesting observations and contributes to the existing knowledge on the subject.

I permit the candidate to submit the work for the award of degree of ***Doctor of Philosophy*** in ***Zoology*** of the ***Aligarh Muslim University, Aligarh, India.***

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Erfanullah

Dedicated To My Father

GENERAL INTRODUCTION

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The success of a commercially viable aquaculture system depends, to a great extent, on a sound knowledge of nutrition of the concerned fish species Experimental studies in fish nutrition are generally undertaken to achieve three important objectives Firstly, to workout the gross nutrient requirements of the species, leading to incorporation of a minimum level of particular nutrient (s) in the test diet, resulting in optimum growth, a phenomenon which is of prime concern to a culturist Secondly, to translate this information into successful artificial dietary formulations, using plant and animal origin feedstuffs Thirdly, to reduce the operating cost on feeding practices involved in the culture system

Fish raised in a relatively small confinement at high stocking density (intensive culture) must receive the required nutrients and energy in the form of protein and amino acids, fat and fatty acids, and carbohydrate In addition, non-energy nutrients, minerals and vitamins, are also adequately required to support life and promote growth (Halver, 1976)

Over the last one decade considerable progress has been made in fish nutrition, and data are available on quantitative nutrient requirements in a number of fish species (Wilson, 1991, NAS - NRC, 1993, and De Silva and Anderson, 1995) However, compared to studies on protein and amino acids (Wilson and Halver, 1986, Cowey, 1988, Pandian, 1989, and Wilson, 1989), fat and fatty acids (Greene and Selivonchick, 1987, Benitez, 1989, and Sargent *et al.*, 1989), vitamins (Halver, 1989, and Tacon, 1991), minerals (Lall, 1989, and Davis and Gatlin, 1991) and energy (Smith, 1989[a]), studies on carbohydrate nutrition are limited

Carbohydrates are considered an inexpensive source of energy in animal production Fish exhibit varying ability to adapt and utilize carbohydrates to obtain energy (Phillips *et al.*, 1966), storing energy reserves as glycogen in liver and muscle (Wendt, 1964), and converting it to fat in the body (NAS - NRC, 1983) Inclusion of carbohydrates improves the binding capacity of compounded diet, besides considerably reducing its cost Like lipid, the use of higher levels of carbohydrate as energy source in formulated diets has a protein sparing effect (Lovell, 1989, and Cho and Kaushik, 1990)

Information so far available on carbohydrate utilization in many fish groups is considerably varied, and as such no specific carbohydrate requirement has been established, owing to the fact that utilization and availability of this nutrient is affected not only by its level, source and complexity, but also by inherent differences relating to the carbohydrate metabolizing enzymes in different groups of fish (NAS - NRC, 1981, 1983 , Jauncey, 1982 , Millikin, 1982, New, 1986 , Cowey, 1988, and Tacon, 1990) Moreover, carbohydrates are merely used to provide energy, and do not supply any essential nutrient (s) that cannot be obtained or synthesized from other dietary nutrients (protein and fat) in a feed (Piper *et al.*, 1989)

However, there is enough experimental evidence to show that both coldwater as well as warmwater fishes are able to utilize varying levels of carbohydrates Fishes like salmonids and yellowtail are reported to be inferior in carbohydrate utilization
(Austreng *et al.*, 1977, Edwards *et al.*, 1977 , Bergot, 1979 [a,b] , Shimeno *et al.*, 1979, 1980, Refstie and Austreng, 1981, Hilton and Atkinson, 1982 , Buddington and Hilton, 1987 , Alsted, 1991, and Furuichi and Yone, 1980), although the former are able to derive some of the energy for growth purposes

and show better nitrogen retention and protein utilization in presence of carbohydrates Similarly, in plaice (Cowey *et al.*, 1975), sea bass (Vaque, 1988), and red seabream (Furuichi and Yone, 1971), incorporation of carbohydrate in the diet results in improved growth and feed conversion efficiencies Carbohydrate utilization is also reported to be high in white sturgeon (Hung *et al.*, 1989, 1990, and Fynn-Aikins *et al.*, 1992, 1993) It has been pointed out that utilization of carbohydrate is influenced by a number of factors such as dietary protein (Furuichi and Yone, 1971, 1982 , Bergot, 1979[a,b], and Hilton and Atkinson, 1982) and fibre content (Shiau *et al.*, 1989) in the diet, feeding rate (Hilton *et al.*, 1987), meal frequency (Murai *et al*, 1983, Tung and Shiau, 1991), body size (Tung and Shiau, 1993), and supplementation of growth promoting substances (Shiau and Chen, 1993, and Shiau and Lin, 1993) It has also been suggested that the capability of a species to adapt to higher levels of carbohydrate in the diet depends on its ability to convert excess energy to lipid or non-essential amino acids (Tacon, 1990) Kaushik *et al* (1989[a]) maintained that the ability of fish to utilize carbohydrate may have both digestive and metabolic origin The poor carbohydrate utilization is attributed to poor glucose tolerance resulting from the lack of insulin response (Palmer and Ryman, 1972 , and Furuichi and Yone, 1981), low insulin receptor activity (Plisetskaya *et al.*, 1986), and low phosphorylating activity (Walton and Cowey, 1982)

Channel catfish and European eel utilize carbohydrate as energy source as effectively as other energy yielding components of the diet (Garling and Wilson, 1977, Dupree and Huner, 1984, Robinson and Lovell, 1984 , Wilson and Poe, 1987 , El-Sayed and Garling, 1988 , de la Higuera *et al.*, 1989 , Garcia- Gallego *et al.*, 1993[a,b], Hidalgo *et al.*, 1993 , and Sanz *et al.*, 1993)

Omnivorous tilapia and carp tolerate elevated levels of dietary carbohydrates (Takeuchi *et al*, 1979[a] , Furuichi and Yone, 1980 , Shimeno *et al*, 1981, Ufodike and Matty, 1983, Viola and Arieli, 1983 , Anderson *et al*, 1984, Zietler *et al*, 1984, Akand, 1986 , and Tung and Shiau, 1991, 1993) Among carp disparity, however, exists on the extent of carbohydrate utilization Jauncey (1982), and Furuichi *et al.* (1987) have shown that carps are able to grow well on diets containing low levels of carbohydrate Luquet and Moreau (1990), Jantrarotai *et al.* (1992), and Nijhof and Bult (1994) have reported that *Clarias* catfish and turbot efficiently utilize non-protein energy from carbohydrates However, in mullet and cod, increased energy consumption derived from carbohydrate did not alter fish growth (Alexis and Papapareskeva-Papoutsogloue, 1986, and Hemre *et al.*, 1989) So far, information on carbohydrate utilization in Indian cultivable finfishes is limited (Sen *et al*, 1978 , and Erfanullah and Jafri, 1993 ,1994)

Similarly, utilization of different carbohydrate sources also considerably vary with fish species Salmonids are unable to utilize complex carbohydrate such as starch and dextrin as compared to simple sugars (Buhler Halver, 1961 , Bergot, 1979[a] , and Pieper and Pfeffer, 1980) Recent studies indicate that glucose is not utilized effectively by salmonids as a source of energy (Hilton and Atkinson, 1982 , Dixon and Hilton, 1985 , Beamish *et al*, 1986 , Buddington and Hilton, 1987, and Hilton *et al*, 1987) Whereas, channel catfish do not make use of glucose, maltose or sucrose as energy source, although complex carbohydrates, such as dextrin and corn starch, are excellently utilized by this fish (Dupree, 1966 , and Wilson and Poe, 1987) Furuichi and Yone (1982) have noted that ability to utilize glucose and dextrin was inferior to that of α - starch in red seabream The growth rate and feed efficiency of carp were highest on α- starch, followed by dextrin and glucose diets (Furuichi and Yone, 1982) Murai *et al.* (1983) have reported the influence of glucose chain length of various carbohydrates (glucose, maltose, dextrin or α-starch) and frequency of feeding on their utilization in fingerling carp At twice daily feeding, α-starch diet resulted in the best weight gain and feed efficiency Improvement in dietary glucose and maltose utilization by carp with increased feeding frequency by atleast 4 times daily was observed Further, as the frequency of feeding increased from 4 to 6 times day^{-1}, weight gains improved together with increased feed intakes in all dietary treatments, however, deteriorations of feed efficiency were noted in such dietary groups and the longer the glucose chain length, the poorer the feed efficiency Buddington (1987) has noted that, unlike trout, carp are able to adapt to quantity, and apparently also to type of digestible carbohydrate, and the adaptive capabilities of their intestinal nutrient transport processes are matched to potential variation in carbohydrate content of the natural diet Shikata *et al* (1994) have investigated the effects of dietary glucose, fructose and galactose on hepatopancreatic enzyme activities and body composition of carp Shimeno *et al* (1979) have shown that, compared to starch, fructose is not efficiently used by yellowtail Tilapia fed glucose containing diet tend to grow slower than those fed dextrin and starch diets (Anderson *et al*, 1984 , and Tung and Shiau, 1991, 1993) Chuang and Shiau (1993) have investigated the effect of different carbohydrate sources on the intestinal disaccharidase activity, plasma glucose level, body composition, and growth of tilapia Feeding tilapia with different carbohydrate sources (glucose, maltose, sucrose, lactose or starch) failed to influence the intestinal disaccharidase activity However, growth rates were high with starch diet followed by disaccharides and glucose diets

Sturgeon fed either maltose or glucose diets had the highest percent energy retained, followed by those fed either dextrin, raw corn starch or cellulose diets (Hung *et al.*, 1989 , and Hung and Fynn-Aikins, 1993) Practically nothing is known about the utilization of different carbohydrate sources in Indian cultivable finfish species

It has been shown by several workers that processing of complex carbohydrate sources can considerably improve carbohydrate utilization in terms of protein and energy retention (Luquet and Bergot, 1976 , Bergot, 1979[a,b] , Bergot and Berque, 1983 , Kaushik and Oliva-Teles, 1985, and Pfeffer *et al.*, 1991) Recently, Bergot (1993) indicated the beneficial effect of cooked starch (pregelatinized or extruded) over native/raw starch in terms of improved starch digestibility in rainbow trout Starch digestibility was significantly higher in Atlantic salmon fed diet containing 45% pre-extruded after-meal (a protein-rich wheat by-product) than 45% pre-extruded whole wheat or 45% crude whole wheat (Arnesen and Krogdahl, 1993) In sturgeon, utilization of raw starch was observed to be poorer than that of pre-treated starch or corn (Kaushik *et al.*, 1989[b]) Spannhof and Kunhe (1977) found lower digestibility values for natural starch of potato and grain in eel, but digestibility of carbohydrate increased considerably when starch was treated hydrothermally and gelatinized However, in the same fish species, other complex carbohydrates, like cellulose and soybean meal, were poorly digested even after heat treatment (Schmitz *et al.*, 1984) Degani *et al.* (1986) have shown that eel grew better on diets containing equal amounts of bread or wheat meal than soluble starch, sorghum or potato starch Furuichi *et al* (1987) have pointed out that, in carp and red seabream, utilization of ß-starch is higher, in terms of growth, feed and protein efficiency, than α-starch

Similarly, in red seabream, diets containing extruded carbohydrate ingredients produced higher weight gain, feed and protein efficiency than those containing raw ingredients (Jeong *et al.*, 1991) In a series of experiments conducted on carp, rainbow trout fingerling, juvenile striped jack and yellowtail, a mixture of raw (ß-) and gelatinized (α-) starch at different dietary energy levels resulted in increased growth, feed and protein efficiency (Jeong *et al.*, 1992[a,b], Takeuchi *et al.*, 1992, and Arakawa *et al.*, 1993) Takeuchi *et al.* (1994) and Hernandez *et al.* (1994) have indicated the nutritive value of gelatinized corn meal as a carbohydrate source in grass carp, hybrid tilapia and common carp Jollivet *et al.* (1988) reported the influence of dietary level and physical state of starch on the apparent nutrient(s) digestibility, amylase secretion, and gastric evacuation in turbot fed different rations and reared at different temperatures Recently, in rainbow trout, Bergero *et al.* (1993) have investigated the effectiveness of alternative energy sources by substituting a part of protein content of diet with starch from raw and processed maize, on *"in vivo"* and *"in vitro"* digestibility and ammonia excretion

Inclusion of appropriate levels of non-protein energy sources in the diet ensures efficient protein utilization (Steffens, 1981 , and Wilson and Halver, 1986) Carbohydrates and fats are the major non-protein energy sources in fish diet Compared to fat, carbohydrates are relatively inexpensive and more readily available source of energy to many fish species Increased levels of dietary lipid may reduce feed intake and assimilation, preventing the intake of necessary amounts of protein and other nutrients for maximum growth, besides resulting in poor pelleting, and rancidity on storage (Jauncey, 1982) It may also adversely affect fish carcass/body composition (Takeuchi *et al*, 1978[b], Millikin, 1983, Piper *et al.*, 1989 , and Hanley, 1991) Excess dietary carbohydrate in feed may also lead to fat deposition in fish by stimulating the activities of lipogenic enzymes (Lin *et al*, 1977 , Likimani and Wilson, 1982 , Hung *et al*, 1989, and Tung and Shiau, 1991) It is thus imperative to work out for the species concerned the optimum carbohydrate-to-lipid ratio (CHO L) that produces the best growth, feed conversion, nutrient retention and body composition Studies on channel catfish (Garling and Wilson, 1977), tilapia (El-Sayed and Garling, 1988) and hybrid striped bass (Nematipour *et al*, 1992) indicate that carbohydrate and lipid are effectively utilized as energy source, and can be substituted for one another at a rate of 2 25 1 commensurate with CHO L physiological fuel values In a recent study on different CHO L ratio, Shimeno *et al* (1993) have noted that feeding high carbohydrate to tilapia accelerates glycolysis and lipogenesis and decelerates gluconeogenesis and amino acid degradation in the liver, indicating high ability of the fish to utilize dietary carbohydrate, and to spare protein for growth Similarly, hybrid *Clarias* catfish (*Clarias marcocephalus X C gariepinus*) has been reported (Jantrarotai *et al*, 1994) to perform equally well on isonitrogenous (33% CP) and isocaloric (281 kcal, DE /100 g) diet, containing carbohydrate from broken rice, forming 37 to 50% of the diet, with lipid ranging from 9 6% to 4 42 (CHO L ratio of 3 82 - 11 24) Almost no information is available on these aspects for Indian cultivable finfish species

Since protein constitutes the single most expensive item in artificial fish feeds, it is logical to incorporate only that amount which is necessary for normal growth Any excess is considered wasteful, biologically as well as economically Knowledge on the optimal protein requirement, and protein sparing effect of non-protein nutrients, such as lipid and carbohydrate, may be useful in reducing feed costs (Cho and Kaushik, 1990 , Kaushik and Cowey, 1991; and Sanz *et al.* 1993). The protein sparing effect of carbohydrate has earlier been established in salmonids (De Long *et al.*, 1958; Buhler and Halver, 1961 , Ringrose, 1971; Lee and Putnam, 1973 ; Bergot, 1979[a,b], Pieper and Pfeffer, 1979 ; and Rychly, 1980), plaice (Cowey *et al.*, 1975), turbot (Adron *et al.*, 1976), sea bass (Alliot *et al.*, 1979 ; and Tandler and Beamish, 1980), yellowtail (Shimeno *et al.*, 1985), channel catfish (Tiemeier *et al.*, 1965 , Page and Andrews, 1973 ; Garling and Wilson, 1977 ; Likimani and Wilson, 1982, and Strange, 1984), European eel (Degani, 1987[a] ; Degani and Viola, 1987; Hidalgo *et al.*, 1993; and Sanz *et al.*, 1993), tilapia (Jauncey and Ross, 1982 , Anderson *et al.*, 1984; Cisneros *et al.*, 1984 ; and Shiau and Peng, 1993), and carp (Chiou and Ogino, 1975; Ogino *et al.*, 1976 ; Shimeno *et al.*, 1981 , and Watanabe *et al.*, 1987).

Information on carbohydrate metabolism and energy kinetics in fish is also limited Although necessary enzymes for digestion and utilization of carbohydrates occur in fish, the role of dietary carbohydrate, and energy contribution of glucose to energy requirements of fish remain unclear (Piper *et al.*, 1989) Several studies, however, indicate that hormonal and metabolic regulations of carbohydrate and energy metabolism in fish vary with species (Cowey and Sargent, 1972, 1979 ; Tarr, 1972 ; Shimeno *et al.*, 1981 , Shimeno, 1982 , Likimani and Wilson, 1982 ; Buddington and Hilton, 1987, Degani, 1987[b] . Hilton *et al.*, 1987 ; Wilson and Poe, 1987 ; Cowey, 1988 , Hung *et al.*, 1989 , Pereira *et al.*, 1991; and Rueda *et al.*, 1993).

The knowledge of nutrient/energy availability from various feedstuffs is felt desirable so that effective substitution of one ingredient by the other may be achieved while formulating least-cost practical diet for fish The important task in evaluating the potential of any feedstuff for inclusion in diet is the measurement of its digestibility (Hastings 1969 , Cho and Slinger, 1979 , Cho *et al.*, 1982,1985 , Cho and Kaushik, 1990, and Cho, 1993) Digestibility estimates also determine the level of indigestible nutrients voided, accounting for a major portion of aquaculture waste Numerous studies in the past were focussed to assess the nutrient(s)/energy digestibility in a number of fish species (Inaba *et al.*, 1963, Pandian, 1967, Lovell, 1977, Andrews *et al.*, 1978, Austreng, 1978 , Cho and Slinger, 1979, Pandey and Singh, 1980 , Singh and Pandey, 1980 , Pfeffer, 1982 , Cho *et al.*, 1982 , Schmitz *et al.*, 1982, 1984, Bergot and Berque, 1983 , Dabrowski, 1983 , De Silva and Perera, 1984 , Ellis and Smith, 1984 , Law, 1984, 1986 , Ash, 1985, Hajra, 1985 , Law *et al.*, 1985 , Wilson and Poe 1985 , Henken *et al.*, 1985, Kirchgessner and Schwarz, 1982, Kirchgessner *et al.*, 1986 , Ferraris *et al* , 1986 , Hanley, 1987 , De Silva,1989 , De Silva *et al.*, 1988, 1990, Hossain and Jauncey, 1989 , Lorico-Querijero and Chiu, 1989, Kamarudin *et al.*, 1989 , Singh, 1992 , Arnesen and Krogdahl, 1993, Hajen *et al.*, 1993[a,b]; Schwarz and Kirchgessner, 1993 , Khan, 1994, Kaushik *et al.*, 1994, and Shiau and Liang, 1994) The significance of using different methods for digestibility measurment in fish have also been looked into by some workers (Hickling, 1966 , Austreng, 1978 , Bowen, 1978, 1981 , Cho and Slinger, 1979 , Buddington, 1980, Lied *et al.*, 1982 , De Silva and Perera, 1983 , Grabner, 1984 , Lall *et al.*, 1984, Tacon and Rodriguez, 1984, De La Noue and Choubert, 1986, De Silva *et al.*, 1990 , Bazoco *et al.*, 1993 , Wetherbee and Gruber, 1993 ,You *et al.*, 1993, Dimes and Haard, 1994, Dimes *et al.*, 1994[a,b], and Galetto and Bellwood, 1994)

Indian major carps, the prime cultivable freshwater fishes, are raised in India on simple supplementary dietary combinations of plant and animal origin feedstuffs (Jhingran, 1991) Intensive cultivation of these fishes is hampered mainly because of the non-availability of balanced rations formulated on the basis of their nutritional requirements The importance of such studies on major carps has been emphasized by several workers (Sinha, 1991 , Alagarswami, 1992, Dehadrai, 1992 , Tripathi, 1992 , and Shetty and Varghese, 1993) Although some information have been made available in the recent past on the gross protein and amino acid requirements (Jayaram, 1978 , Renukaradhya and Varghese, 1986 , Singh and Bhanot, 1988 , De Silva and Gunasekera, 1991 , Khan and Jafri, 1991[a] , 1993 , Ravi and Devaraj, 1991, Khan *et al.*, 1993[a], and Das *et al.*, 1994), little is known about dietary carbohydrate utilization in these fishes (Sen *et al.*, 1978 , and Swamy *et al*, 1990)

Catfish farming is of recent origin in India The growing interest in the cultivation of catfishes in India has, however, created the need to investigate the basic nutritional requirements of these fishes to formulate cost-effective feeds (Dehadrai, 1980, 1992, Jhingran, 1991, and Sinha, 1991) Significant achievements have been made in evolving viable culture techniques for air-breathing catfishes, mainly, *Clarias batrachus* and *Heteropneustes fossilis* (Thakur, 1991) Lack of detailed knowledge on nutritional requirements of these fishes remains a major constraint in the development of their intensive culture Information on the nutrition of these fishes seem confined to their protein (Chuapoehuk, 1987 , Boonyaratpalin, 1988 , Akand *et al.*, 1989, Khan and Jafri, 1990, 1991[b] , and Firdaus, 1993), lipid (Boonyaratpalin, 1988 , and Anwar and Jafri, 1992, 1995), and vitamin (Sitasit *et al.*, 1984, and Butthep *et al.*, 1985) requirements Other studies relating to nutrition of these fishes included assessment of energy and protein maintenance requirements (Hassan and Jafri, 1994), optimum energy to protein ratio (Tanomkiate, 1984), optimum ration/meal size (Reddy and Katre, 1979 , and Pakulska *et al.*, 1986), feeding frequency (Marian *et al.*, 1982), and growth and haematology (Firdaus *et al.*, 1994) Almost no information is available on dietary carbohydrate utilization in these commercially important catfish species

As stated earlier, information on carbohydrate utilization with respect to its level, source, optimum CHO L ratio, digestibility, and its protein-sparing action are almost lacking in Indian cultivable fishes This study was, therefore, undertaken with a view to generate data on the above subject, and the observations are presented in the form of this thesis

The thesis consists of two parts Part *I* contains information on effect of dietary carbohydrate level, source, optimum CHO L ratio, and protein-sparing action of dietary carbohydrates, on the growth, conversion efficiency, nutrient retention and body composition in some selected cultivable finfish species, namely, the Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*), stinging catfish (*Heteropneustes fossilis*) and the walking catfish (*Clarias batrachus*)

Part *II* embodies data on dry matter, nutrient and energy digestibility coefficients of carbohydrate rich feedstuffs in the Indian major carps

The study, in addition to contributing to the existing knowledge on the subject, provides data which would be of interest to fish nutritionists, feed technologists, farmers and fishery managers engaged in research and culture of the above species

GENERAL METHODOLOGY

GENERAL METHODOLOGY

I. Source and acclimation of test fish

Induced bred fry and fingerling of Indian major carps, *Catla catla, Labeo rohita* and *Cirrhinus mrigala*, were obtained from the Uttar Pradesh Fish Seed Cooperative Production Centre, Kolahar, Mathura, India These were transported to the laboratory in oxygen filled polythene bags, given a prophylactic dip in $KMnO_4$ solution (1 3000), and stocked in earthen pond (12 x 6 x 1 5 m) During this period, supplementary feeding at a total rate of 5% of body weight was carried out with conventional feed mixture (rice bran and mustard oil cake, 1 1) at 0800 and 1600 h Required number of fishes were transferred to wet laboratory, disinfected with $KMnO_4$ and acclimated for two weeks on casein-gelatin H-440 test diet (Halver, 1976) near to satiation in 70 l high density polyvinyl circular troughs (water volume 55 l), covered with a nylon mesh and fitted with a continuous flow-through system (1-1 5 l/min), with 75% of the trough surface darkened by a black polyethylene sheet

Young catfishes, *Heteropneustes fossilis* and *Clarias batrachus*, were procured from local ponds, disinfected with $KMnO_4$ (1 3000), and stocked in flow-through (1-1 5 l/min) outdoor cement cisterns (3 x 1 2 x 1 m) The fishes were fed to satiation low-quality minced meat Required number of fish were transferred to wet laboratory and acclimated as above on H-440 test diet

II. Formulation of experimental diets

Experimental diets were formulated by slightly modifying the procedure used by Buhler and Halver (1961) Casein, gelatin and fish meal were used as protein sources The protein content in the diets was fixed according to the requirement of the species worked out earlier in this laboratory (Anonymous, 1991) Dextrin and bread-flour were used as dietary carbohydrate sources for studying the effects of varying levels of carbohydrate on growth, conversion efficiency, body composition, and nutrient retention in fish The required level of carbohydrate in the diets was achieved by varying dextrin and/or bread-flour component (s) at the cost of α-cellulose A mixture of corn and codliver oil (2 1) was used as fat Vitamin and mineral premixes (Table I & II) were prepared according to Halver (1976) Carboxymethyl cellulose was employed as binder

In the experiment designed for evaluating the effects of different carbohydrate sources on growth, conversion efficiency, body composition, and nutrient retention in fish, equal quantities of different carbohydrate sources (glucose, maltose, fructose, sucrose, dextrin, corn-starch and α-cellulose) were incorporated in the experimental diets

For studying the effects of varying carbohydrate-to-lipid ratio, dietary dextrin and lipid were selectively interchanged in the diet at a rate of 2 25 1, to commensurate with carbohydrate-to-lipid physiological (ME) values

In the experiment designed for evaluating the protein sparing effects of different carbohydrate sources, isocaloric experimental diets, containing three levels of protein, were formulated At each protein level, inclusion of three levels of the selected carbohydrate source (glucose, sucrose and dextrin) was made

Estimates of apparent nutrient and energy digestibility coefficient of carbohydrate rich feedstuffs were made for Indian major carps, using a reference (40% crude protein, 1% chromic oxide as external marker) and the test diets (Cho *et al.*, 1985)

III Testing the palatability and stability of experimental diets

Before conducting the feeding trial, characteristics like palatability and water stability of the experimental diets were tested in various forms viz, soft cake and dry crumbles Water stabilıty of diets was investigated based on analysis of change in dry weight over a 3 hour period by immersing diets in experimental troughs without fish All the feeds had a stability of above 94% in 3 hours The method of preparation of these diets were as follows

i. Soft cake

Calculated quantities of air-dry ingredients were weighed on a top pan electronic balance (Precisa 120 A) A measured quantity of water was taken in a stainless steel attachement of Hobart electric mixer and heated (75 - 80°C) Gelatin was dissolved into it with slow stirring and heating the content onto a double boiler unit The mixer bowl was removed from heating, dextrin and mineral mix added to it, and the content blended in Hobart mixer while still in lukewarm state This was followed by the addition of remaining ingredients, excepting carboxymethyl cellulose which was added in the last As the diet began to solidify, the speed of the stirring was increased to incorporate air into the mix The final diet, with the consistency of bread dough, was poured into a teflon-coated pan and placed into a refrigerator to gel The prepared diet was in the form of moist cake (50% dry matter) from which small cubes were cut and kept in sealed polythene bags in a freezer (- 20°C) until used

ii. Dry crumbles

For the preparation of dry crumbles, dough obtained as above was cold extruded through a Hobart extruder fitted with 1 mm die The strands were spread over the receiving tray and placed in hot air oven (60°C) The diet upon drying was crumbled and sieved through ASTM-18, 30 and 500 μm screens, and stored in sealed polythene bags (4°C)

IV. Feeding trial

Fish of the desired size and number were sorted out from the acclimated lots and stocked, in triplicate groups, in 70 l flow-through (1 - 1 5 l/min) polyvinyl circular troughs (water volume 55 l), for each dietary treatment Fish were fed the experimental diets twice daily at 0800 and 1600 h, six days a week, near to an apparent satiation The feeding schedule was chosen after carefully observing the feeding behaviour and intake of the fish Accumulation of the diet at the bottom was avoided to prevent leaching of the nutrients Any uneaten food was siphoned immediatly over a filter screen, dried in a hot air oven, and reweighed to measure the amount of total food consumed The total feed offered each day was recorded for calculating the food conversion (dry to wet basis) Each morning, before commencement of feeding, faecal matter was siphoned off from the experimental troughs For length and weight measurements, fish were anaesthetized with MS-222 (tricane methanesulfonate, 1 10,000) and measured at the beginning of the experiment as well as at weekly intervals Fish were bulk weighed on a sensitive top pan balance (Varbal, VB/TLA, India and/or Precisa, 120 A) No feed was given on the day of weekly measurement A thorough scrubbing and cleaning of each trough was carried out every week A natural photoperiod (light dark) was maintained during the experiments At the end of the feeding trial, fish were randomly sacrificed with an overdose of MS-222 solution, and kept in freezer (-20 $^\circ$C) for the assessment of carcass/body composition

V. Physico-chemical conditions of the rearing water

Water temperature ($^\circ$C) was recorded with a portable water analysis kit (Naina, India) while dissolved oxygen (mg/dl or ppm) estimated following Winkler's modified technique (APHA, 1985) The measurements were made daily at 0800 and 1600 hours

VI. Proximate analysis

Estimation of proximate composition in representative sample of feed ingredients, experimental diets and fish were made using the standard AOAC (1984) techniques All chemical analysis were based on triplicate runs

i. Estimation of moisture

5 - 10 g of finely ground/homogenized sample was taken in a pre-weighed silica crucible and placed in hot air oven (105 ± 1 $^{\circ}$C) for 24 hours The crucible containing the dried sample was cooled in a desiccator and reweighed The loss in weight was expressed as percentage of moisture

ii. Estimation of ash

Dried powdered sample (2 - 5 g) was taken in a pre-weighed silica crucible and incinerated in a muffle furnace (600 $^{\circ}$C) for 2 - 4 hours till the sample became completely white and free of carbon After cooling in a desiccator, the content was reweighed to estimate the amount of ash as per cent of dried sample

iii. Estimation of crude fat

Crude fat was extracted by continuous soxhlet extraction technique using petroleum ether (40 - 60 $^{\circ}$C B P) as solvent Finely ground dried sample (2 - 3 g) was placed in Whatman fat extraction thimble, plugged with cotton, and introduced into the soxhlet A clean, dry, pre-weighed soxhlet receiver flask was fitted to the soxhlet assembly for extraction Extraction was carried out for 10 - 12 hours At the end of extraction, the solvent was reclaimed and the flask removed and kept in a hot air oven (100 $^{\circ}$C) to remove the traces of solvent It was then transferred to a desiccator, cooled and reweighed The difference between the weight of the flask before and after the extractıon gave the quantıty of crude fat extracted from the known amount of sample The result was expressed as per cent dry weıght

iv. Estimation of crude protein

Estımatıon of crude proteın was made by slıghtly modıfyıng the Wong's mıcro-kjeldhal method, as adopted by Jafrı *et al.* (1964) The prıncıple ınvolves dıgestıng the sample wıth N-free sulphurıc acıd ın presence of a catalyst, potassıum persulphate, whıch converts the nıtrogenous compounds to ammonıum sulphate Thıs ıs then nesslerızed and the colour developed, due to the formatıon of complex compound (NHg_2I), ıs measured spectrophotometrıcally The optıcal densıty ıs read agaınst a standard curve of $(NH_4)_2SO_4$ for nıtrogen estımatıon from whıch the amount of crude proteın ıs calculated

A known amount of drıed, finely ground sample (0 1 - 0 5 g) was taken ın a kjeldahl flask contaınıng 5 ml of sulphurıc acıd (1 1) and heated tıll fumes dısappeared After coolıng, 0 5 ml of saturated potassıum persulphate was added to oxıdıze the dıgestıng mıxture The dıgestıon was contınued for about 12 - 16 hours tıll the solutıon became water clear, ındıcatıng that all the nıtrogenous materıal present ın the sample has been converted ınto ammonıum sulphate After coolıng, the dıgested mıxture was transferred to a 50 ml volumetrıc flask and raısed to mark wıth double dıstılled water An alıquot of thıs solutıon (0 5 ml) was taken ın a dry test tube, and 0 1 ml each of sulphurıc acıd (1 1) and saturated potassıum persulphate solutıon added to ıt The volume was raısed to 3 ml wıth double dıstılled (deıonızed)water The solutıon was then nesselerızed wıth 7 ml of Bock and Benedıct's Nessler reagent (Oser, 1971) and kept at room temperature for 10 mın for complete colour development A blank was prepared sıde by sıde, substıtutıng the alıquot wıth dıstılled water The absorbance was measured, after settıng the ınstrument wıth a blank, at 480 *nm* The amount of nitrogen was obtained by reading the optical density against a standard calibration curve (Fig 1) prepared by plotting the absorbance values against graded concentration of ammonium sulphate The amount of total nitrogen was multiplied with conventional protein factor (6 25 X N) to obtain the crude protein content in the sample The values were calculated as per cent dry weight

v. Estimation of crude fibre

Crude fibre was quantified as loss on ignition of dried residue, remaining after digestion of sample with standard solution of sulphuric acid and sodium hydroxide, under carefully controlled conditions A weighed quantity of dry, powdered and fat-free sample (2 - 4 g) was taken in a spoutless conical flask fitted to a reflex condenser Boiling distilled water was added to it, followed by addition of 25 ml sulphuric acid (1 25 w/v), the content mixed, and its volume raised to 200 ml, and boiled for 30 min An antifoam agent (octyl alcohol) was added to prevent bumping during boiling The solution was removed from heating and the residue collected over a Whatman # 541 filter paper using Buchner funnel fitted to a suction pump The residue was washed at least three times with boiling water It was then transferred to a beaker and to this was added sodium hydroxide (1 25 w/v), and the volume raised to 200 ml with boiling water Boiling was continued for another 30 min At the end of boiling, the mixture was filtered through a porous crucible and the collected residue washed with boiling water and 1% hydrochloric acid, and again with boiling water To remove traces of water, the residue was washed with methanol, and dried to a constant weight at 100 $^{\circ}$C This was then allowed to cool in a desiccator, weighed and incinerated for 2 - 3 hours in a muffle furnace (600 $^{\circ}$C) The crucible containing the incinerated sample was transferred to a desiccator, cooled and reweighed The difference between the weight of the residue after drying and ignition denoted the amount of fibre contained in the sample, and the value expressed as per cent dry weight

vi. Calculation of nitrogen-free extract

Nitrogen-free extract was determined indirectly, subtracting the sum of moisture, crude protein, crude fat , ash and fibre (%) from 100

vii. Organic matter

Organic matter in the sample was calculated by subtracting the ash (%) from dry matter (%)

VII. Estimation of energy

i. Gross energy

Estimation of gross energy was made on a ballistic bomb calorimeter (Gallankamp, Loughborough, England) Prior to estimation, a weighed amount of dried powdered sample (0 5-1 0 g) was taken in a metallic crucible and compacted carefully to increase the rate of combustion at 25 lb oxygen pressure The heat generated upon combustion was read on the modulated galvanometer scale, and converted to energy equivalent, worked out earlier using thermochemical grade benzoic acid (6 32 kcal g^{-1}) as a standard The gross energy was expressed as kcal g^{-1}

ii. Metabolizable energy

Metabolizable energy was calculated using physiological fuel values, 4, 4 and 9 kcal g^{-1} for protein, carbohydrate and fat, respectively (Garling and Wilson, 1977)

VIII. Estimation of chromic oxide

Chromic oxide was estimated with wet acid digestion technique (Furukawa and Tsukahara, 1966) Diet and faecal sample (0 50 - 1 0 g), containing chromic oxide as a marker, were separately taken in a kjeldahl flask and digested by heating in 5 0 ml conc nitric acid for about 30 min to remove organic matter Additional nitric acid was added to prevent the content from becoming dry The mixture was removed from heating, allowed to cool, and 3 0 ml of perchloric acid (60%) added to it Heating was resumed till no more fumes evolved, indicating disappearance of all traces of nitric acid Oxidation of chromium oxide to dichromate was indicated by the development of a brilliant golden yellow colour After cooling, the content was washed thoroughly into a 100 ml volumetric flask and the content made to mark with double distilled water After keeping the solution for 10 min at room temperature, for complete colour development, the optical density was measured on a spectrophotometer at 350 *nm* The concentration of chromic oxide in the sample was measured as absorbance against a calibration curve (Fig 2), prepared by plotting absorbance values against graded concentration of chromic oxide The chromic oxide content in the sample was expressed as mg/100 g, dry weight

IX. Estimation of plasma glucose

Plasma glucose was estimated according to the method described by Dubowski (1962) Blood was drawn from the artery/vein complex into a heparinized syringe and stored in heparinized glass vials for analysis To obtain plasma, blood was centrifuged at 8500 x g for 5 min and the aliquot immediately frozen (- 20° C) until analysis

With positive displacement in a piston type pipettor, 0 1 ml of plasma was taken in a test tube and 1 9 ml of 3 0% (w/v) trichloroacetic acid added to it The content was mixed well and allowed to stand for at least 5 min , and centrifuged for 10 min at 2500 rpm Three test tubes were taken and marked separately as, 'Test', 'Blank' and 'Standard' 1 0 ml of the above aliquot was taken into the 'Test' test tube 1 0 ml of distilled water was taken in the 'Blank' test tube while 1 0 ml of working standard glucose solution (0 1 mg/ml) pipetted into the 'Standard' test tube 5 0 ml of O-toluidine reagent was added to these tubes The content was mixed well by careful lateral shaking The tubes were then capped with aluminium foil and kept in a boiling water bath exactly for 10 min Thereafter, these were removed from the water bath, and allowed to cool in cold tap water for at least 4 min The absorbance of the 'Test' and 'Standard' aliquotes was measured at 630 *nm* on a spectrophotometer after setting the instrument with the 'Blank' The plasma glucose concentration (mg/dL) was calculated with the equation

$$\text{glucose mg/dL} = \frac{\text{Absorbance of 'Test' aliquot}}{\text{Absorbance of 'Standard' aliquot}} \times 200$$

All spectrophotometric measurements were made on Milton Roy Microprocessor-modulated splitbeam 1001 spectrophotometer

X. Assessment of growth, conversion efficiency, nutrient retention, and biological indices

Calculation of various growth parameters, conversion efficiency, nutrient retention, and biological indices were made according to the following standard definitions (Wee and Tacon, 1982 , Tabachek, 1986 , Hardy, 1989, and Kim and Kaushik, 1992)

$$\text{Weigth gain (\%)} = \frac{F_n B_w\,(g) - I_n B_w\,(g)}{I_n B_w\,(g)} \times 100$$

$$\text{Specific growth rate}(\%) = \frac{\text{Log}_e\ F_n B_w\ (g) - \text{Log}_e\ I_n B_w\ (g)}{\text{Duration (days)}} \times 100$$

$$\text{Feed intake (mg/fish}^{-1}) = \frac{\text{Total dry feed intake (g)} \div \text{Number of fish trough}^{-1}}{\text{Duration (days)}} \times 1000$$

$$\text{Feed conversion ratio} = \frac{\text{Total dry feed intake (g)}}{F_n B_w\ (g) - I_n B_w\ (g)}$$

$$\text{Protein efficiency ratio} = \frac{F_n B_w\ (g) - I_n B_w\ (g)}{\text{Protein intake (g)}}$$

Nutrient retention efficiency (g^{-1} or $kcal.g^{-1}$) =

$$100 \times \frac{\{(F_n B_w\ (g) \times \%\ \text{nutrient of}\ F_n B_w) - (I_n B_w (g) \times \%\ \text{nutrient of}\ I_n B_w)\}}{\text{Nutrient intake}\ g^{-1}\ \text{or}\ kcal.g^{-1}}$$

$$\text{Hepato-somatic index} = \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}} \times 100$$

$$\text{Gastro-somatic index} = \frac{\text{Gut Weight (g)}}{\text{Total body weight (g)}} \times 100$$

Where, $F_n B_w$ and $I_n B_w$ = Final and initial average body weight , respectively

XI. Statistical analysis

The data was statistically analysed using Central Computer Facility (VAX 11/780) Comparisons among treatment means or between the initial and final values of the same treatment means were made by one way analysis of variance (*ANOVA*), followed by Duncan's Multiple Range Test at 0 05% probability level (Snedecor and Cochran, 1967 , Sokal and Rholf, 1981, and Duncan, 1955) Second degree polynomial regression analysis ($Y = a + bX + cX^2$) was employed to weight gain (%) data to predict maximum weight gain (%) in response to dietary nutrient intake Simple regression ($Y = a + bX$) and correlation coefficient (r) were also calculated to establish the relationship between dietary nutrient intake and growth/body constituents (Snedecor and Cochran, 1967)

Table 1. Vitamin premix*.

Vitamin	g/100 g diet
Alpha cellulose	8 000
Choline chloride	0 500
Inositol	0 200
Ascorbic acid	0 100
Niacin	0 075
Ca pantothenate	0 050
Riboflavin	0 020
Menadione	0 004
Pyridoxine HCl	0 005
Thiamin	0 005
Folic acid	0 0015
Biotin	0 0005
Alpha tocopherol acetate**	0 040
Vitamin B_{12}	10 mg/500 ml H_2O

* Halver (1976)

** Incorporated with oil

Table 2. Mineral premix*.

Mineral	g/100 g diet
Calcium biophosphate	13 580
Calcium lactate	32 700
Ferric citrate	2 970
Magnesium sulphate	13 700
Potassium phosphate (dibasic)	23 980
Sodium biophosphate	8 720
Sodium chloride	4 350
Allumınium chloride $6H_2O$	0 015
Potassium iodide	0 015
Copper chloride	0 010
Manganous sulphate H_2O	0 080
Cobalt chloride $6H_2O$	0 100
Zinc sulphate $7H_2O$	0 300

* Halver (1976)

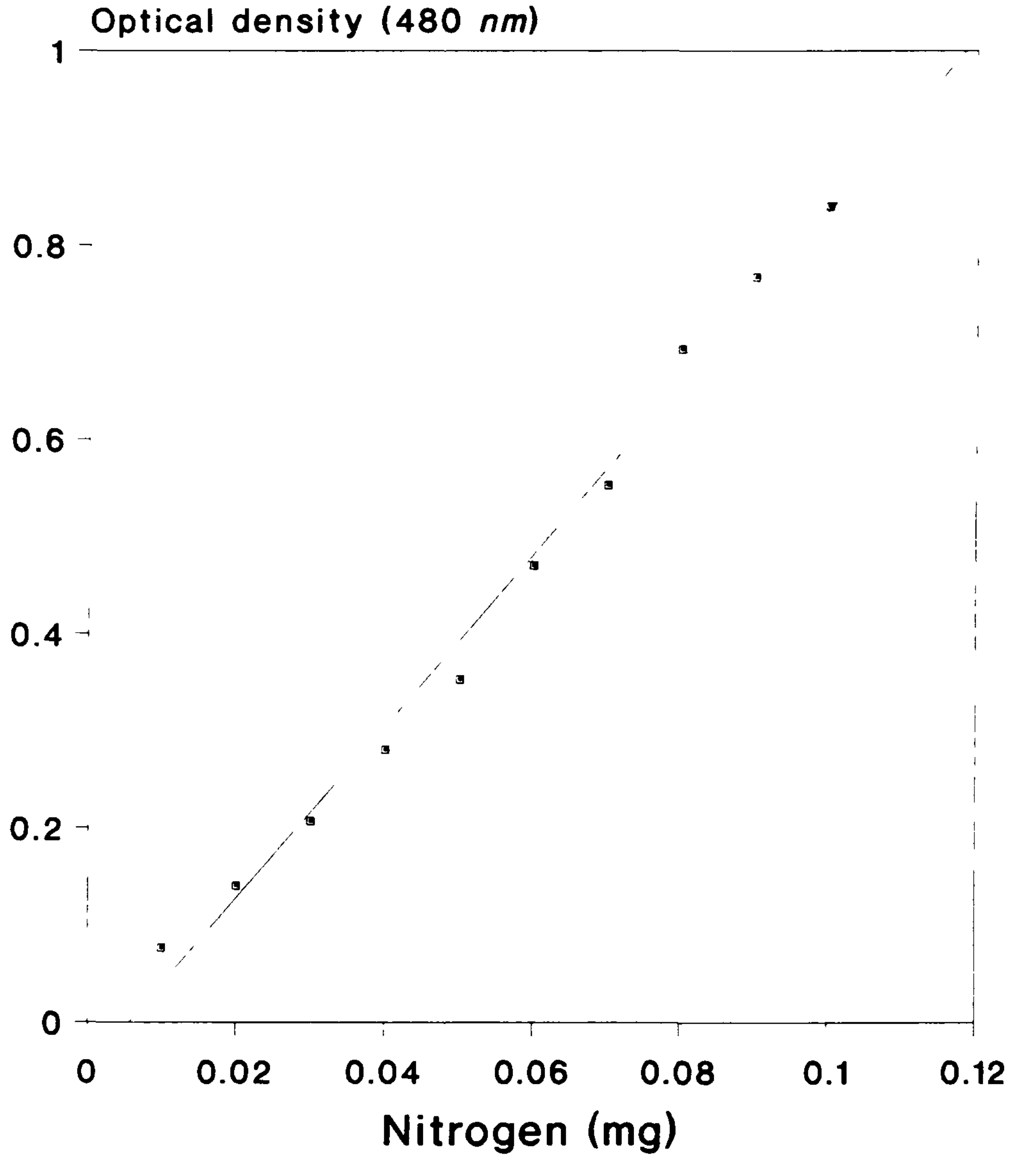


Fig.1. Calibration curve of nitrogen

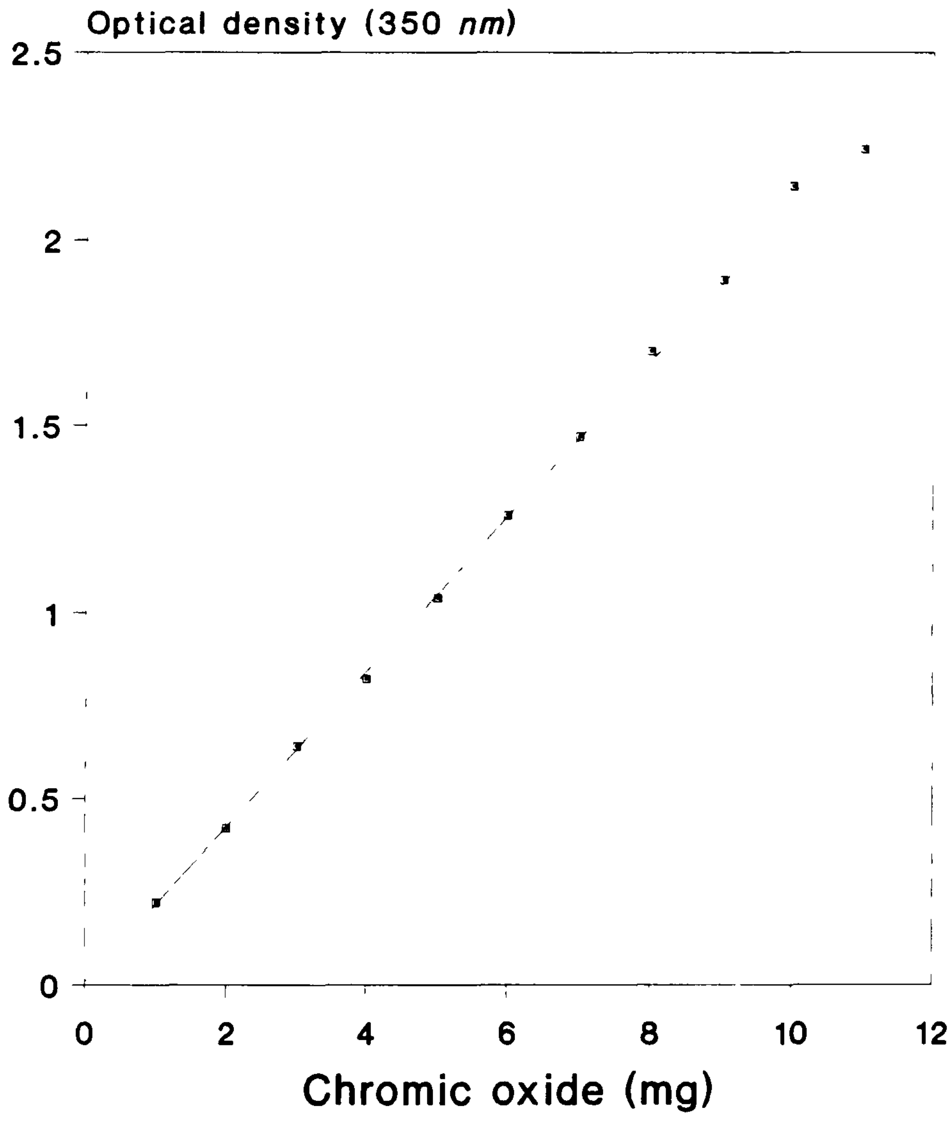
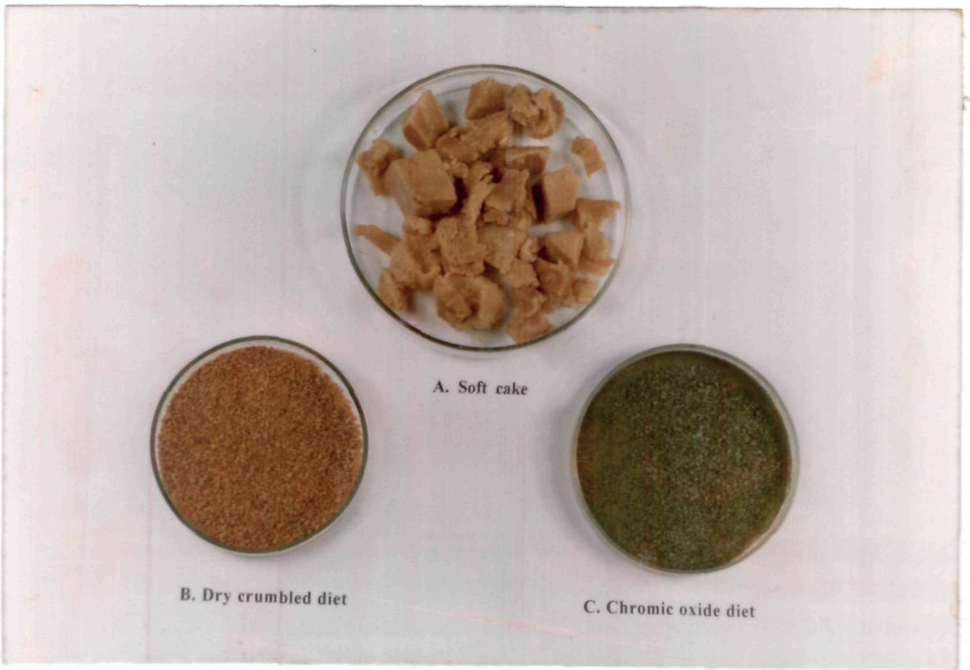


Fig. 2. Calibration curve of chromic oxide

Flow - through system used for feed trials



Experimental diets used for feed trials

Fish being acclimatized on experimental diet

Part I

Chapter 1

CHAPTER - I

GROWTH RESPONSE OF YOUNG CATFISH, *HETEROPNEUSTES FOSSILIS* (BLOCH), FED VARYING LEVELS OF DIETARY CARBOHYDRATE

INTRODUCTION

Carbohydrates are relatively cheap and inexpensive source of dietary calories in formulated fish feeds Besides being an immediate source of dietary energy, carbohydrates also serve as precursor for various intermediary metabolic functions (NAS-NRC, 1983) The inclusion of carbohydrate component in fish feed improves its pelling quality and nutritive value (Lovell, 1989)

Fish exhibit varying ability to adapt and utilize carbohydrate for energy purposes (Buhler and Halver, 1961, and Phillips *et al.*, 1966), storing energy reserves as glycogen in the liver and muscle (Wendt, 1964), and converting excess calories to fat in the body (NAS-NRC, 1983) In many fish species, information on carbohydrate utilization are varied, owing to the fact that utilization and availability of this nutrient is affected not only by its level, source and complexity, but also by inherent differences relating to enzymatic patterns in different fish groups (NAS-NRC, 1981, 1983, Jauncey, 1982, Millikin, 1982, Cowey, 1988, and Tacon, 1990) Unlike protein and fat, carbohydrate as a nutrient is not considered essential to fish because of their ability to synthesize carbohydrate metabolites (glucose/glycogen, etc) from excess dietary calories obtainable from protein/fat Inclusion of feedstuffs with relatively high levels of carbohydrate in formulated fish feed is, however, preferred in veiw of its protein sparing action which makes the diet cost-effective (Hidalgo *et al.*, 1993, and Sanz *et al al.*, 1993)

H. fossilis, an omnivorous air-breathing catfish, is widely distributed throughout the Indian sub-continent, inhabiting swampy and marshy water bodies The fish has a high marketability due to its superior nutritive value, and is generally propagated on extensive lines (Pillay, 1990, Jhingran, 1991, and Thakur, 1991) Lack of information on its nutritional requirements is a major constraint in the development of intensive culture of this species Information on the nutrition of this species seems limited only to its protein requirment (Akand *et al.*, 1989, and Firdaus, 1993), and carbohydrate and lipid utilization (Akand *et al* , 1991, and Anwar and Jafri, 1992) This study reports the growth, conversion efficiency, nutrient retention, and body composition of young *H. fossilis* fed varying levels of dietary carbohydrate

MATERIALS AND METHODS

Experimental diets

Seven different iso-nitrogenous (40% CP), casein-gelatin based semi-purified diets containing graded levels of carbohydrate (0 - 30%), were formulated (Table 1) Dextrin was used as digestible carbohydrate source, and its level varied to obtain the desired level of carbohydrate by substituting with α-cellulose A mixture of corn and codliver oil (2 1) was used as lipid The vitamin and mineral premixes used were according to Halver (1976) Dietary crude protein content in the experimental diets was fixed according to the requirment of the species (Firdaus, 1993). Gross energy (kcal.g^{-1}, GE) content in the diets was determined on Gallenkamp ballistic bomb calorimeter Metabolizable energy (kcal.g^{-1} ,ME) in the diet was calculated using the physiological fuel values (page 19). The GE and ME in the experimental diets ranged from 3.35 to 4.5 and 2.42 to 3.62 kcal.g^{-1}, respectively. The energy to protein ratio (as ME) in the diets varied from 6.03 to 9.02 kcal.g^{-1} protein

Method of preparation of the experimental diet has been described under General Methodology Section (page 14). Proximate composition of the diets were estimated according to standard methods (page 16-19).

Feeding trial

Source of fish, their acclimation, and details of general experimental design have been given in General Methodology (page 12, 15).

H. fossilis (10.80±1.7 cm; 8.15 ± 0.23 g) were sorted out from a previously acclimated fish lot maintained on casein-gelatin based semi-purified H-440 test diet (Halver, 1976) in wet laboratory, and randomly stocked in triplicate groups of 10 fish each in 70 l high density polyvinyl flow-through (1 - 1 5 l/min) type indoor circular troughs (water volume 55 l) Fish were fed the experimental diets to apparent satiation in the form of moist cake, six days a week, twice daily at 0800 and 1600 hours. Feeding trial was conducted for six weeks. Initial and weekly weight gains (g) were recorded on a sensitive top pan balance (Varbal, India), after anaesthetizing the fish with MS 222 solution (1:10,000). Average water temperature and dissolved oxygen, based on daily measurements, over the experimental period were 27 ± 1°C and 6 6 ± 0 2 ppm, respectively.

Growth parameters and feed utilization efficiencies were measured using standard definitions (page 21-22)

Proximate composition and gross energy analysis

Before commencement of the feeding trial, ten fish were randomly sacrificed with an overdose of MS 222 solution, and pooled sample, in triplicate,taken for the determination of initial wholebody composition, and for the assessment of hepato- and gastro-somatic indices using standard methods (page 16-19, 22) At the end of the feeding trial, five fish from each dietary treatment were likewise sampled for their final body composition and biological indices Wholebody energy was determined on Gallenkamp ballistic bomb calorimeter (page 19)

Statistical analysis

Comparisons among different treatment means or between initial and final values of the same dietary treatment were made by one-way analysis of variance (*ANOVA*) and Duncan's multiple range test (P< 0 05) Simple regression and corrletaion coefficient (r) were calculated to establish the relationship between dietary carbohydrate intake (X) and growth/nutrient retention efficiency/body constituents (Y)

RESULTS

The data indicate that, over the period of feeding trial, weight gain (%), SGR (%), PER, protein and energy retention efficiencies increased significantly (*ANOVA* P < 0 05) up to 20% dietary carbohydrate incorporation, thereafter, a distinct fall (P < 0 05) in these parameters was noticeable (Table 2) The relationship between the above levels of carbohydrate intake (0-20%) with weight gain(%), SGR(%), PER and protein retention efficiency were found to be linear and positive (Fig 1 - 2) Energy retention efficiency also registered a positive relationship (Y=101 64 + 1 552 X, r=0 95, P < 0 05, N=15) up to the above carbohydrate inclusion in the diet FCR registered a linear negative relationship (Y = 1 942 - 0 0366 X , r= - 0 958, P < 0 05, N=15) with carbohydrate up to 20% inclusion level in the diet The fat retention efficiency, on the other hand, maintained a linear positive relationship (Y = 43 587 + 2 115 X, r = 0 994, P<0 05, N = 21) up to the maximum level of carbohydrate (30%) tested

Changes in body composition of fish revealed a marked influence of dietary carbohydrate (Table 3) With increase in carbohydrate intake from 0 to 30 %, organic matter, crude fat and gross energy (kcal g^{-1}) increased significantly (P < 0 05) over the initial, and between the dietary treatments Crude protein and inorganic matter (ash) was not affected (P > 0 05) while moisture content varied inversely with the levels of carbohydrate intake The relationships between dietary carbohydrate intake (0 to 30 %) and different body constituents have been given in Table 4

A four-fold increase in liver and gut weights (g), and a two-fold increase in hepato- and gastro-somatic indices were observed in fish fed 30% carbohydrate (Table 3)

DISCUSSION

In most fish, optimum utilization of dietary carbohydrate is reported to be within the range of 10-30% (Cowey and Sargent, 1972, Furukawa, 1976, Edwards *et al*, 1977, Metailler *et al*., 1980, Furuichi *et al*., 1986, Degani 1987[a]; Berger and Halver, 1987 , Fynn-Aikins *et al.*, 1992 , and Arakawa *et al*,1993) Tilapia and European eel are perhaps the only exceptions where levels of carbohydrate in the diet as high as 40 - 44% do not supress growth (Anderson *et al.*, 1984, Akand, 1986, and Hidalgo *et al.*, 1993) Warmwater fishes as such do not have a true carbohydrate requirement (NAS-NRC, 1983), although incorporation of certain level of carbohydrate in their diet influence overall growth and conversion efficiencies The results on *H fossilis* indicate a strong positive linear relationship between dietary carbohydrate intake, within a range of 0 - 20%, corresponding to 2 42 to 3 23 kcal g^{-1} (ME), and fish growth, expressed as live weight gain/SGR The finding seems to conform with observations on chinook salmon (Buhler and Halver, 1961), red seabream (Furuichi and Yone, 1980), rainbow trout (Edwards *et al*, 1977, Hilton and Atkinson, 1982), yellowtail (Furuichi *et al.*, 1986), and walking catfish fry (Mollah and Alam, 1990) In cod, however, it was reported that weight gain and retention values for protein and fat were not influenced when amount of dietary carbohydrate was raised from 0 - 30 % (Hemre *et al*, 1989) It has been suggested that the capability of a fish species to adapt to higher carbohydrate content in the diet depends on its ability to convert excess energy to lipid or non-essential amino acids (Tacon, 1990) Relatively low growth, poor feed and nutrient retention efficiency noted in *H. fossilis* fed carbohydrate-free or low carbohydrate (5 - 10%) diets could be attributed to insufficient non-protein energy sources in the diet, necessitating greater utilization of dietary protein for purposes other than growth (maintenance and basal metabolism) Since such diets also contained higher levels of indigestible fibre in the form of α-cellulose, less efficient absorption and reduced availability of other necessary nutrients appear understandable

Depressed growth and poor feed efficiency obtained with diets containing levels of carbohydrate higher than the optimum (20%) point to the fact that this fish is unable to handle higher carbohydrate level in the diet Reduction in fish growth beyond the above level of carbohydrate may also be attributed to altered energy to protein ratios in the diet The ratios varied from 8 55 to 9 05 kcal g^{-1} protein in diets containing 25 and 30% carbohydrate, respectively A proper balance between energy (ME) and protein is necessary for better growth and feed efficiency Evidently, in *H. fossilis*, diet with 20% carbohydrate and 3 23 kcal g^{-1} (ME), corresponding to energy to protein ratio of 8 08 kcal g^{-1}protein, produced maximum growth and best feed efficiency In brook trout, maximum weight gain and feed efficiency have been reported at almost similar energy to protein ratio (8 0 kcal g^{-1} protein) in the diet (Ringrose, 1971) Besides other factors, nutrient composition and energy density of diet also influence growth rate and feed conversion in fish Best FCR (1 3) in *H fossilis* was obtained at optimum (20%) dietary carbohydrate Improved FCR values at optimum dietary carbohydrate was similarly seen in salmonids (Buhler and Halver, 1961, and Edwards *et al.*, 1977), yellowtail, red seabream (Furuichi and Yone, 1980), European eel (Degani and Viola, 1987) and walking catfish fry (Mollah and Alam, 1990) On the other hand, no significant difference was seen in feed gain values reported for rainbow trout (Hilton and Atkinson, 1982) and white sturgeon (Fynn-Aikins *et al.*, 1992) fed increasing levels of dietary cerelose or glucose

It has been stated that dietary energy content and its source also strongly influence protein utilization in fish (Steffens, 1981) The strong positive linear relationship observed between carbohydrate intake (from 0 - 20%) and PER or protein retention efficiency point to a beneficial effect of carbohydrate inclusion as an important non-protein energy source in the diet of *H fossilis* With increase in dietary carbohydrate from 0 - 20%, the percent calorie contribution from this nutrient increased from 0 - 25% (ME), with consequential decline in protein calories from 66 - 50% This increment in carbohydrate calories at the expense of protein calories improved weight gain, SGR, PER and nutrient retention efficiency, suggesting towards an effective protein sparing action of dietary carbohydrate Sparing action of dietary carbohydrate on protein has similarly been noted in salmonids (Buhler and Halver, 1961, Shimeno *et al.*, 1979, and Pieper and Pfeffer, 1980), turbot (Adron *et al.*, 1976), plaice (Cowey *et al.*, 1975), channel catfish (Garling and Wilson, 1976), seabass (Alliot *et al.*, 1979), and more recently in European eel (Hidalgo *et al*, 1993, and Sanz *et al.*, 1993) In these fishes, increased PER occurred up to optimum dietary carbohydrate utilization Cod, however, appears to be an exception where PER has been shown to decline with carbohydrate intake (Hemre *et al.*, 1989)

Existence of a strong positive relationship between dietary carbohydrate intake and body constituents of *H. fossilis* demonstrates that body composition gets altered with levels of carbohydrate fed, just as has been pointed out for other fish species (Buhler and Halver, 1961, Pieper and Pfeffer, 1980, Refstie and Austreng, 1981, Hilton and Atkinson, 1982, Furuichi and Yone, 1982, Anderson *et al.*, 1984, Degani and Viola, 1987, Fynn-Aikins *et al.*, 1992, and Kim and Kaushik, 1992) In channel catfish, however, increased dietary dextrin reportedly fail to increase the amount of carcass lipid (Dupree, 1969, and Garling and Wilson, 1976)

Although protein and energy retention efficiencies were maximum in fish at 20% dietary carbohydrate, no significant ($P > 0.05$) change in retention efficiency values could be seen beyond this level Interestingly, fat retention efficiency continued to increase with the level of carbohydrate inclusion in the diet Again, the strong positive correlation between dietary carbohydrate intake, within 0 - 20% of diet, and percent protein, fat or energy retention efficiency clearly emphasizes the fact that this omnivorous catfish is able to efficiently handle carbohydrate only up to 20% inclusion, converting excess carbohydrate (> 20%) into body lipid Likimani and Wilson (1982), and Brauge *et al.* (1993) have attributed increased lipid deposition in the body of channel catfish and rainbow trout fed high carbohydrate diet to lipogenic enzyme activity In several fish species, nutrient retention efficiency is reported to increase with optimum dietary carbohydrate intake (Buhler and Halver, 1961, Pieper and Pfeffer, 1980, Anderson *et al.*, 1984, Kaushik and Oliva-Teles, 1985, Degani and Viola, 1987, Kim and Kaushik, 1992, and Hidalgo *et al.*, 1993) However, in fishes like salmonids (Edwards *et al*, 1977, Shimeno *et al*, 1979, and Bergot, 1979[a]), white sturgeon (Fynn-Aikins *et al*, 1992), and cod (Hemre *et al.*, 1989), no significant effect of dietary carbohydrate on percent protein and/or energy retention was observed

Higher values of gastro- and hepato-somatic indices, noted in the fish with increasing levels of dietary carbohydrate, were not found to affect growth, conversion efficiencies and protein or energy utilization Gross external anatomy of liver also did not reveal any discernable change with carbohydrate intake, although carbohydrate contained diets enhanced fat deposition around the gut, contributing to increased gut weight Earlier findings on other fishes (Buhler and Halver, 1961, Lee and Putnam, 1973, Edwards *et al.*, 1977, Bergot, 1979[a], Furuichi and Yone, 1980, Hilton and Atkinson, 1982, and Fynn-Aikins *et al.*, 1992) provide a corollary to this observation It can thus be inferred from the present study that, in a 40% CP and 3 23 kcal g^{-1} ME, inclusion of 20% carbohydrate, corresponding to an energy to protein ratio of 8 08 kcal g^{-1} protein, produces maximum growth, best conversion efficiencies and higher protein utilization in young *H. fossilis* However, inclusion of excess carbohydrate (> 20 %) which lead to progressive fat deposition in carcass is undesirable as it affects the quality of the fish

SUMMARY

Effects of varying levels of dietary carbohydrate have been examined on the growth, conversion efficiencies, body composition, and nutrient retention efficiency of young catfish *H. fossilis* (10 80±1 7 cm, 8 15±0 23 g) Iso-nitrogenous (40% CP) experimental diets, containing different levels of carbohydrate (0,5,10,15,20,25 or 30%), with variable gross (3 35 - 4 50 kcal g^{1}) and metabolizable (2 41- 6 32 kcal g^{-1}) energy, and energy to protein ratio (6 03 to 9 02 kcal g^{-1} protein as ME), were fed to triplicate groups of ten fish each in 70 l high density polyvinyl flow-through (1-1 5 l/min) type indoor circular troughs (water volume 55 l) Fish were fed to apparent satiation, six days a week, twice daily at 0800 and 1600 h Over the 6-week growth trial, maximum growth, conversion efficiencies, and increased nutrient retention were seen in fish at 20% dietary carbohydrate and 3 23 kcla g^{-1}ME, corresponding to an energy to protein ratio of 8 08 kcal g^{-1} protein Changes in the wholebody composition were significantly *(P 0.05)* affected by the levels of carbohydrate intake Increase in dietary carbohydrate resulted in higher values for dry matter, fat and energy contents Crude protein and ash remained unaffected *(P 0.05)*, while moisture decreased *(P 0.05)* with increase in dietary carbohydrate levels Protein and energy retention efficiencies increased linearly up to 20% carbohydrate inclusion in the diet beyond which a significant fall *(P 0.05)* in the values appeared However, fat retention continued to increase progressively with carbohydrate intake A four-fold increase in liver and gut weights, and a two-fold increase in hepato- and gastro-somatic indices were observed in fish fed 30% dietary carbohydrate

Table 1. Ingredient and proximate composition of expiremental diets.

	Dietary carbohydrate levels (%)						
(g/100g, as fed)	0	5	10	15	20	25	30
Basal premix[1]	66.22	66.22	66.22	66.22	66.22	66.22	66.22
White dextrin	0.00	5.00	10.00	15.00	20.00	25.00	30.00
α-cellulose	33.78	28.78	23.78	18.78	13.78	8.78	3.78
	Proximate composition (% dry weight)*						
Crude protein	40.10	40.11	40.03	40.11	40.00	40.07	40.12
Crude fat	9.10	9.04	9.01	9.03	9.00	9.06	9.00
Ash	7.24	7.33	7.30	7.24	7.32	7.28	7.24
Crude fibre	43.56	38.32	32.96	28.32	23.24	18.58	13.50
Nitrogen-free extract[2]	Nil	5.20	10.70	15.30	20.44	25.01	30.14
Gross energy[3]	3.35	3.55	3.74	3.93	4.12	4.31	4.50
Metabolizable energy[4]	2.42	2.62	2.84	3.03	3.23	3.42	3.62
% ME distribution (P:C:L)[5]	66:0:34	61:8:39	56:15:29	53:20:27	50:25:25	47:29:24	44:33:23

[1]Casein, 38.09 (84% CP); gelatin, 9.13 (87.6% CP); corn oil, 6.00; codliver oil, 3.00; vitamin premix, 1.00;mineral premix, 4.00 and carboxymethyl cellulose, 5.00 g/100g, respectively.

* Mean of triplicate runs ± SEM; *N*=3.

[2] By difference.

[3] Based on determined values on ballistic bomb calorimeter. GE contributed by a-cellulose and CMC was subtracted, so that GE levels from ingredients are more truly represented.

[4] Based on physiological fuel vaues, 4.00, 4.00 and 9.00 kcal g^{-1} for protein, carbohydrate and lipid, respectively (Garling and Wilson, 1977). [5] (P : C : L)= protein, carbohydrate and lipid, respectively.

Table 2. Growth, survival, conversion and nutrient retention efficiencies in *H. fossilis* fed varying levels of dietary carbohydrate*

	Dietary carbohydrate levels (%)						
	0	5	10	15	20	25	30
Initial individual wet weight, g	7.90 ±0.06	7.96 ±0.04	7.90 ±0.06	7.90 ±0.06	7.80 ±0.06	7.85 ±0.06	7.81 ±0.08
Final individual wet weight, g	13.25^{a} ±1.28	14.97^{b} ±1.54	15.80^{bc} ±1.52	17.50^{d} ±1.32	17.80^{d} ±1.46	16.24^{c} ±1.25	16.00^{c} ±1.32
Weight gain (%)	67.22^{a} ±12.43	88.10^{b} ±15.11	101.30^{c} ±15.11	121.52^{d} ±12.40	128.20^{d} ±14.23	107.00^{c} ±11.71	104.90^{c} ±11.95
Specific growth rate (%)	1.23^{a} ±0.23	1.50^{b} ±0.17	1.67^{bc} ±0.16	1.90^{d} ±0.12	1.96^{d} ±0.14	1.73^{c} ±0.12	1.70^{c} ±0.12
Protein efficiency ratio	1.22^{a} ±0.12	1.49^{b} ±0.17	1.65^{bc} ±0.17	1.87^{d} ±0.11	1.90^{d} ±0.16	1.71^{c} ±0.13	1.69^{c} ±0.12
Feed intake (mg/ fish day^{-1})	259.29^{a} ±10.11	280.95^{b} ±17.72	288.33^{bc} ±16.13	306.25^{d} ±17.11	308.33^{d} ±10.12	294.05^{c} ±17.41	288.10^{c} ±16.11
Food conversion ratio	2.04^{d} ±0.14	1.68^{c} ±0.10	1.53^{bc} ±0.17	1.33^{a} ±0.17	1.30^{a} ±0.14	1.47^{b} ±0.12	1.48^{b} ±0.12
Survival (%)	95.00	98.00	98.00	99.00	99.00	98.00	98.00
Nutrient retention efficiency (%)							
Protein	26.65^{a} ±6.04	31.86^{b} ±9.70	32.84^{b} ±11.70	37.74^{c} ±9.60	39.34^{c} ±12.70	35.40^{b} ±11.00	34.74^{b} ±11.15
Fat	37.60^{a} ±9.50	48.08^{b} ±12.40	71.09^{c} ±12.40	83.53^{d} ±10.30	92.32^{e} ±11.30	96.13^{e} ±15.70	98.48^{e} ±12.40
Energy	97.14^{a} ±9.80	112.61^{b} ±16.80	120.14^{c} ±15.10	127.35^{c} ±14.74	128.58^{c} ±10.80	110.43^{b} ±12.44	106.06^{b} ±11.63

* Mean of triplicate runs ± SEM; N=30 fish each/dietry treatmentValues in the same row not sharing the same superscripts are significantly different ($P < 0.05$)

Table 3. Body composition, liver and gut weight, hepato- and gastro-somatic indices of *H. fossilis* fed varying levels of dietary carbohydrate (% wet weight)*

	Dietary carbohydrate levels (%)							
	Initial#	0	5	10	15	20	25	30
Moisture[1]	79.15	75.39^{e}	74.70^{e}	73.19^{d}	72.03^{c}	71.78^{bc}	71.14^{ab}	70.90^{a}
	±0.21	±0.23	±0.40	±0.28	±0.32	±0.43	±0.38	±0.52
Crude protein	14.28	17.34^{a}	17.65^{a}	17.21^{a}	17.52^{a}	17.80^{a}	17.67^{a}	17.53^{a}
	±0.19	±0.17	±0.12	±0.12	±0.18	±0.18	±0.16	±0.24
Crude fat	1.81	3.90^{a}	4.38^{b}	5.83^{c}	6.34^{d}	6.97^{e}	7.22^{f}	7.60^{g}
	±0.05	±0.08	±0.12	±0.10	±0.17	±0.13	±0.11	±0.16
Ash	3.06	3.19^{a}	3.04^{ab}	3.15^{a}	3.63^{b}	3.05^{a}	3.28^{a}	3.21^{a}
	±0.08	±0.06	±0.12	±0.05	±0.11	±0.13	±0.06	±0.10
Nitrogen-free extract[2]	1.70	0.18^{a}	0.23^{ab}	0.62^{e}	0.48^{cd}	0.40^{c}	0.69^{ef}	0.76^{f}
	±0.15	±0.12	±0.15	±0.12	±0.12	±0.17	±0.16	±0.16
Gross energy[1]	5.03	5.54^{a}	5.65^{b}	5.81^{c}	5.84^{cd}	5.93^{e}	5.95^{e}	6.01^{ef}
	±0.002	±0.003	±0.01	±0.004	±0.003	±0.003	±0.006	±0.007
Liver weight, g	0.09	0.18^{a}	0.22^{b}	0.26^{c}	0.36^{d}	0.39^{d}	0.38^{d}	0.37^{d}
	±0.01	±0.01	±0.02	±0.02	±0.02	±0.03	±0.03	±0.04
Gut weight, g	0.18	0.35^{a}	0.44^{b}	0.55^{c}	0.69^{d}	0.75^{e}	0.73^{e}	0.74^{e}
	±0.01	±0.02	±0.01	±0.03	±0.04	±0.06	±0.04	±0.04
Hepato-somatic index	1.13	1.36^{a}	1.47^{b}	1.65^{c}	2.06^{d}	2.20^{e}	2.34^{f}	2.31^{f}
	±0.02	±0.03	±0.04	±0.05	±0.07	±0.08	±0.09	±0.10
Gastro-somatic index	2.25	2.64^{a}	2.93^{b}	3.44^{c}	3.87^{d}	4.29^{e}	4.50^{f}	4.63^{fg}
	±0.08	±0.07	±0.09	±0.08	±0.07	±0.07	±0.01	±0.02

* Mean of triplicate runs ±SEM; N=15 fish each/dietary treatment; #=10 fish

Values in the same row not sharing the same superscripts are significantly *($P<0.05$)* different

[1] Dry weight basis

[2] By difference

Table 4. The relationship between dietary carbohydrate (X) and body constituents (Y) in H. fossilis.

Body constituents (% wet weight)	Relationship	r	n	p<
Dietary carbohydrate to				
Moisture	**Y=75.09-0.157X**	**-0.968**	**21**	**0.05**
Dry matter	**Y=24.91+0.157X**	**0.968**	**21**	**0.05**
Organic matter	**Y=21.74+0.154X**	**0.978**	**21**	**0.05**
Crude protein	**Y=17.40+0.0086X**	**0.457**	**21**	**n.s.**
Crude fat	**Y=4.11+0.128X**	**0.971**	**21**	**0.05**
Inorganic matter (ash)	**Y=3.17+0.0031X**	**0.170**	**21**	**n.s.**
Gross Energy (Kcal g^{-1})	**Y=5.59+2.152X**	**0.968**	**21**	**0.05**

n.s., not significant

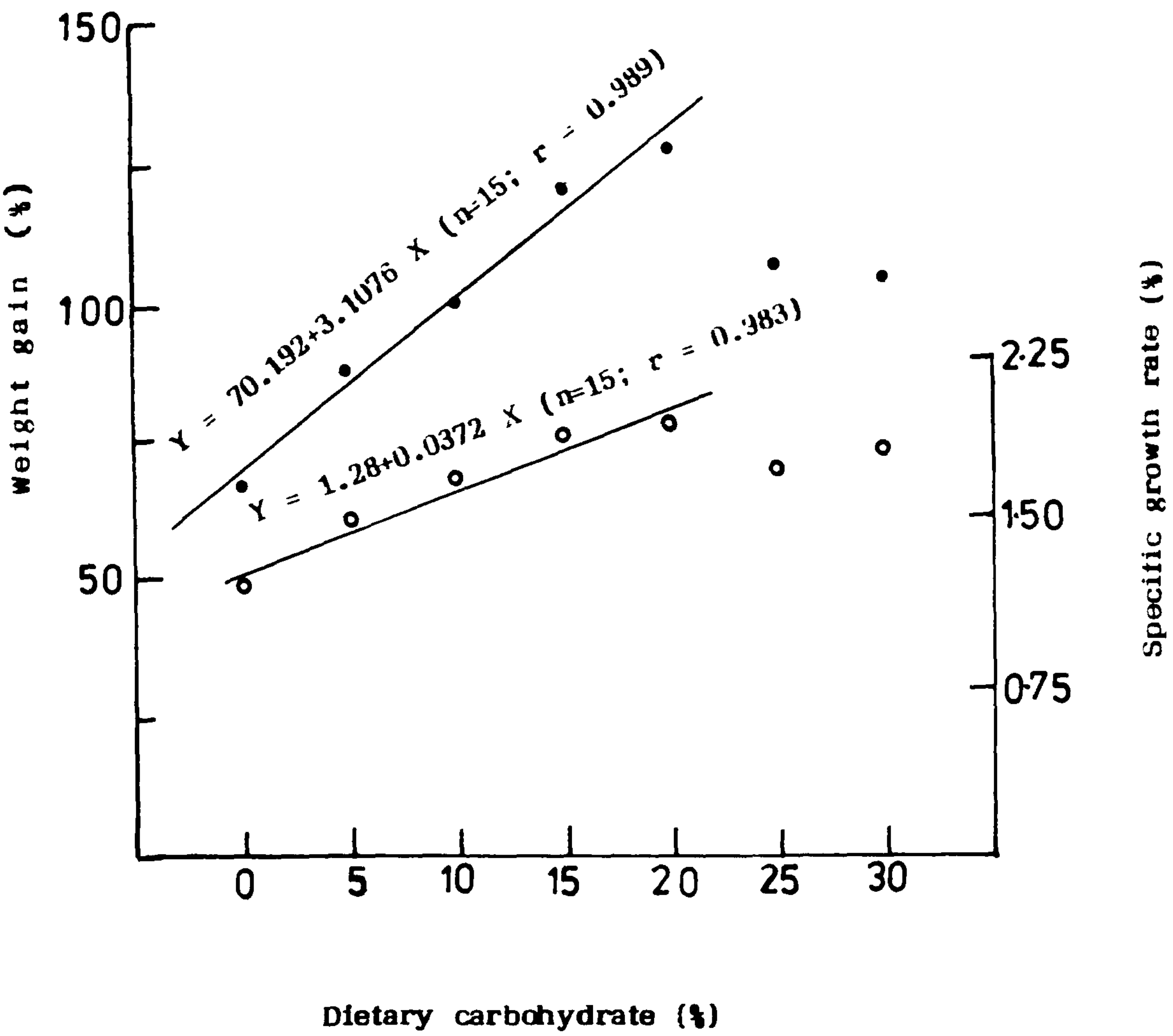


Fig. 1. Effect of increasing levels of dietary carbohydrate on weight gain (●) and specific growth rate (O) in *H. fossilis*.

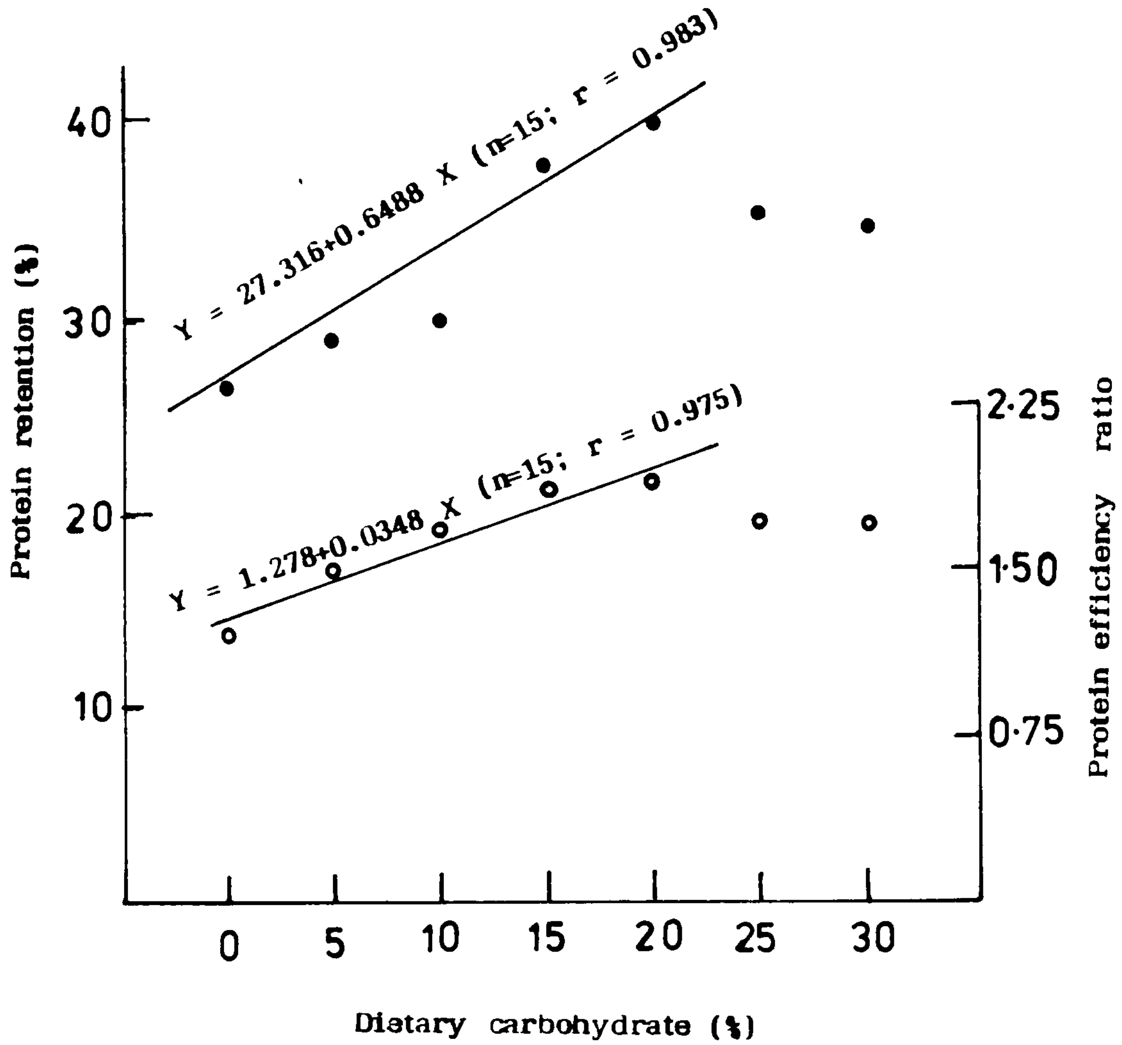


Fig. 2. Effect of increasing levels of dietary carbohydrate on protein retention (●) and protein efficiency ratio (O) in *H. fossilis*.

Chapter II

CHAPTER II

GROWTH, FEED CONVERSION, BODY COMPOSITION, AND NUTRIENT RETENTION EFFICIENCY IN FINGER-LING CATFISH, *HETEROPNEUTES FOSSILIS* (BLOCH), FED DIFFERENT SOURCES OF DIETARY CARBOHYDRATE

INTRODUCTION

The varying ability of different fish species to optimally handle a particular level of-dietary carbohydrate, and to efficiently use this nutrient, has been attributed to several factors such as protein level (Luquet *et al* , 1975, and Bergot, 1979 [a,b,c]), non-protein component (Garling and Wilson, 1977, Wilson and Halver, 1986, Medale *et al.*, 1991, and Shiau and Peng, 1993), degree of gelatinization, and complexity of carbohydrate in diet (Buhler and Halver, 1961, Hilton and Atkinson, 1982, Bergot and Berque, 1983, Murai *et al.*, 1983, Kaushik and Oliva-Teles, 1985, Degani *et al.*, 1986, Furuichi *et al.*,1986, Wilson and Poe, 1987, and Tung and Shiau, 1991,), levels of carbohydrate digestive enzymes (Shimeno *et al.*, 1977, 1979, Hilton and Atkinson, 1982, Hung *et al.*, 1989, and Tung and Shiau, 1991), and the ability of the fish to regulate blood/plasma glucose and insulin concentration (Palmer and Ryman, 1972, and Hilton *et al.*, 1987) More recently, Tung and Shiau (1993) have shown that fish size may also influence carbohydrate utilization

In an earlier study, maximum weight gain, best feed conversion and nutrient retention were obtained in young *H. fossilis*, fed casein-gelatin based 40% CP diet, with 20% carbohydrate (dextrin) and 3 23 kcal g^{-1} (ME), corresponding to energy to protein ratio of 8 08 kcal g^{-1} protein (Chapter *I*) This study reports the growth, feed conversion, body composition, and nutrient retention efficiency in fingerling *H. fossilis* fed different sources of dietary carbohydrate

MATERIALS AND METHODS

Experimental diets

Seven different iso-nitorgenous (40% CP) and almost iso-caloric (4 70 kcal g^{-1}, GE) casein-gelatin based semi-purified diets, containing 20% either of glucose, fructose, maltose, sucrose, dextrin, corn-starch (pre-cooked) or α-cellulose were formulated (Table 1) Crude protein content in the experimental diets was fixed according to the requirement of the species reported earlier from this laboratory (Firdaus, 1993) A mixture of corn and codliver oil (2 1) was used as lipid The vitamin and mineral premixes used were according to Halver (1976) Estimated gross energy in the diet ranged from 4 66 to 4 76 kcal g^{-1} Preparation of the diet has been described under General Methodology Section (page 14) Proximate composition of experimental diets was estimated according to standard methods (page 16-19)

Feeding trial

Source of fish, their acclimation, and details of general experimental design have been given elsewhere (page 12, 15)

H fossilis (6 2 ± 1 0 cm, 3 27 ± 0 04 g) were sorted out from a previously acclimated fish lot maintained on H - 440 test diet (Halver, 1976) in wet laboratory, and randomly stocked in triplicate groups of 20 fish each in

70 l high density polyvinyl flow-through (1 - 1 5 l/min) type indoor circular troughs (water volume 55 l)

Fish were fed the experimental diets (moist cake) to apparent satiation, twice daily at 0800 and 1600 h, six days a week Feeding trial lasted for six weeks Initial and subsequent weekly weight gains (g) were recorded, after anaesthetizing the fish with MS 222 solution (1 10,000) Average water temperature and dissolved oxygen, based on daily measurements, over the experimental period were $29 \pm 1^{\circ}$ C and 6 7 $\pm$ 0 2 ppm, respectively Growth parameters and feed utilization efficiencies were measured using standard definitions (page 21- 22)

Proximate composition and gross energy analysis

Before commencement of the feeding trial, ten fish were randomly sacrificed with an overdose of MS 222 solution, and pooled sample, in triplicate, taken for the determination of the initial wholebody composition, using standard methods (page 16- 19) At the end of the feeding trial, five fish from each dietary treatment (N = 15 fish) were sampled as above for their final body composition Wholebody energy was determined on ballistic bomb calorimeter (page 19)

Plasma glucose level

At the end of the feeding trial, fish were pooled and fasted for 24 hours to allow for the complete evacuation of meals At the end of the fasting period, four fish were randomly selected from each treatment group and their blood sample collected and pooled to represent 0h Thereafter, experimental diets were fed to each group, and four fish from these were again sampled randomly at 1, 2, 3, 4, 5, 6, 7, and 8 h for blood collection At the appropriate sampling intervals, fish were carefully netted from respective troughs and anaesthetized with MS 222 solution (1 10,000) Blood was drawn from caudal artery/vein complex into heparinized syringe, and centrifuged at 8500 x g for 5 min to obtain plasma Plasma glucose level was estimated with O-toulidine method (Dubowski, 1962) Method of plasma glucose estimation has been described elsewhere (page 20 - 21)

Statistical analysis

Comparisons among different treatment means or between initial and final values of the same treatment were made by one-way analysis of variance (*ANOVA*) and Duncan's multiple range test ($P < 0\ 05$)

RESULTS

Percent weight gain, SGR(%), FCR, PER and survival of fingerling *H fossilis* fed different carbohydrate sources have been given in Table 2 Significant ($P < 0\ 05$) differences were noticeable in the growth and feed efficiency of fish with sources of carbohydrate used Maximum weight gain (166 20%) was seen in fish fed dextrin containing diet Weight gain in fish receiving diets with sucrose or pre-cooked corn-starch was not significantly ($P > 0\ 05$) different from each other In comparison to the above carbohydrate sources, glucose, maltose or fructose containing diets produced significantly ($P < 0\ 05$) lower growth The lowest weight gain (85%) was obtained with diet containing α-cellulose as a carbohydrate source

The highest SGR (2 30%) and best FCR (1 5) were noted in fish fed dextrin containing diet, followed by diets containing sucrose, corn-starch, glucose, maltose and fructose, while the lowest SGR (1 45 %) and FCR (3 28) were seen in fish fed α-cellulose based diet

The highest PER (1 67) was observed in fish receiving dextrin, followed by sucrose containing diet No significant ($P > 0\ 05$) difference was evident between fish fed corn-starch and glucose incorporated diets Fish fed α-cellulose containing diet produced the lowest PER (0 77) Survival rate was generally high (98%) in fish receiving different test diets

Variations in the plasma glucose concentrations, in fish receiving different carbohydrate sources, were significant ($P < 0\ 05$) with respect to level (maximum and minimum) and recovery over the sampling time (Fig 1) At the end of the 24 h fasting, the mean plasma glucose concentration was 71 mg/dL Post-feeding glucose and maltose diets resulted in plasma glucose level to a maximum of 206 and 198 mg/dL at 4 h, followed by a steep decrease to 103 and 124 mg/dL, respectively, after 8 h interval Plasma glucose level peaked at 3 h with post-feeding sucrose or fructose diets (141 and 138 mg/dL, respectively), and steadily declined to 112 and 91 mg/dL for post-fed sucrose and fructose diets, respectively Post-feeding dextrin diet also similarly peaked the plasam glucose level within 3 h which remained constant until 5 h It then steeply declined to a level of 82 mg/dL after 8 h period However, post-feeding corn-starch and α-cellulose diets produced relatively low ($P < 0\ 05$) variations in plasma glucose level over the initial (0 h), as well as over 8 h sampling period

Wholebody constituents at the start and end of the experiment, and nutrient retention efficiency were significantly (*P* < 0 05) affected with carbohydrate sources in the diet (Table 3) All experimental groups at the end of the study exhibited significanly (*P* < 0 05) higher percentages of fat, ash and energy, and a lower percentage of moisture, than the fish analysed at the start Fish fed the experimental diets, with the exception of α-cellulose containing diet, tended to accumulate higher body crude protein over the initial Maximum protein retention (33%) was observed with dextrin, followed by corn-starch and sucrose containing diets, while higher fat (115%) and energy (95%) retention values were observed with sucrose based diet Dietary α-cellulose produced significantly (*P*< 0 05) lower values for protein, fat and energy retention

DISCUSSION

Feeding *H. fossilis* with different carbohydrate sources clearly indicate that growth and feed efficiency get altered by the nature of carbohydrate in the diet, and that all carbohydrates are not utilized by the fish with the same efficiency Similar observations were made in salmonids (Buhler and Halver, 1961 , and Buddington and Hilton, 1987), yellowtail (Shimeno *et al* , 1977), channel catfish (Dupree, 1966 , Wilson and Poe, 1987), red seabream (Furuichi and Yone, 1982), cod (Hemre *et al.*, 1989), European eel (Degani *et al* , 1986), sturgeon (Hung *et al.*, 1989), tilapia (Anderson *et al.*, 1984 , and Tung and Shiau, 1991, 1993) and carp (Shimeno *et al.*, 1977 , Furuichi and Yone, 1982 , and Murai *et al.*, 1983)

The ability of fish to utilize carbohydrate may have both digestive and a metabolic origin (Kaushik *et al.*, 1989[a]) The complexity or the structure of dietary carbohydrate and its physical state are known to influence its utilization by fish (Pieper and Pfeffer, 1980 , Spannhof and Plantikow, 1983 , Bergot and Berque, 1983 , Kaushik and Oliva-Teles, 1985 , and Watanabe *et al.*, 1987)

The better performance of diet containing dextrin, pre-cooked corn-starch or sucrose, as observed in *H. fossilis*, indicates that in omnivorous fish utilization of disaccharide and polysaccharide is relatively high This observation is well supported by similar findings on other fish species (Furuichi and Yone, 1982 , Watanabe *et al.*, 1987 , Wilson and Poe, 1987 , and Tung and Shiau, 1991, 1993) Similarly, the poor performance of diets containing glucose, fructose or maltose for this fish is in agreement with studies on other fishes (Furuichi and Yone, 1982 , Hilton and Atkinson, 1982 , Beamish *et al* , 1986 , Buddingon and Hilton, 1987 , Wilson and Poe, 1987 , and Tung and Shiau, 1991, 1993) where it was seen that even most digestible carbohydrate like glucose (> 98%) could not produce increased growth or better feed efficiency It has been explained that in such fishes a large portion of the absorbed glucose probably gets excreted out before adequate insulin is available to facilitate its tissue utilization as an effective energy source (Pieper and Pfeffer, 1980 , Furuichi and Yone, 1981 , Hilton and Atkinson, 1982 , and Buddington and Hilton, 1987) Low insulin level, lack of sensitivity of the hormone (Palmer and Ryman, 1972 , and Thorpe and Ince, 1974) and a relatively low phosphorylating activity in liver lowers glucose utilization in fish (Walton and Cowey, 1982 , and Tung and Shiau, 1991) Alvarado and Robinson (1979), and Hokazono *et al.* (1979) have attributed poor growth performance of fish fed glucose incorporated diet to inhibition of amino acid absorption by the intestine Contrary to the above observations, in rainbow trout (Buhler and Halver, 1961 , and Millikin, 1982), white sturgeon (Hung *et al.*, 1989), and tilapia (Anderson *et al.*, 1984) better growth and feed efficiencies occurred when these fishes received glucose or maltose based diets Surprisingly, no significant differenes were found in the growth of red seabream fed various sources of carbohydrate (Furuichi and Yone, 1982)

Since in all the experimental groups fish readily consumed the offered diet almost instantly, the possibility of leaching of nutrients is removed Hence, the differences observed in the growth parameters of fish fed various of carbohydrates can only be attributed to the nature/source of the dietary carbohydrate

The pattern of changes in plasma glucose concentration in *H. fossilis* fed dextrin or pre-cooked corn-starch diets were similar to that reported for rainbow trout (Bergot, 1979[c]), channel catfish (Wilson and Poe, 1987), and red seabream and common carp (Furuichi and Yone, 1982) It has been opined that the relatively slow rate of digestion of the above carbohydrates (dextrin and pre-cooked corn-starch), and subsequent gradual absorption of plasma glucose to coincide with maximum insulin secretion, account for the maximum utilization of the circulating glucose resulting in increased growth and better nutrient utilization The results on *H. fossilis* with respect to changes in plasma glucose level of fish fed simple sugars (glucose or maltose), in comparison to disaccharides and polysaccharides (sucrose, dextrin or corn-starch), also conform with earlier studies (Furuichi and Yone, 1982 , Furuichi *et al.*, 1986 , Shiau and Lin, 1993, and Tung and Shiau, 1993) Poor absorption of plasma glucose in fish fed fructose containing diet, reported similarly for channel catfish, could be linked to low absorption of fructose from the intestinal tract and low conversion to glucose (Wilson and Poe, 1987) The lack of any discernable change in the plasma glucose level of fish fed diet containing α-cellulose as carbohydrate source was also seen in white sturgeon (Hung *et al.*, 1989)

The pattern of changes in body constituents, over the initial and between the dietary treatments, showed maximum protein accumulation and higher protein retention with dextrin or corn-starch containing diets, suggesting that these carbohydrates are readily utilized by *H. fossilis* for its energy needs, sparing protein calories for growth Fish fed sucrose containing diet, on the other hand, had significantly higher dry matter, crude fat, and fat and energy retention, compared to fish that received the other carbohydrate sources in their diets, indicating towards effective lipogenesis In an earlier experiment conducted at this laboratory on fingerling *L. rohita*, higher carcass lipid content and increased fat and energy retention values similarly occurrred in fish fed 30% surcose in 40% CP diet (Erfanullah, 1991) In white sturgeon fed maltose or glucose diets (Hung *et al.*, 1989), and in tilapia (Tung and Shiau, 1991) fed glucose, dextrin or starch diets, six times a day, higher body lipid accumulation was seen to occur, and this hyperlipedimia and lipogenesis was ascribed to higher lipogenic activities in fish

Low body crude protein, crude lipid, energy and nutrient retention values in *H. fossilis* fed fructose, maltose or glucose containing diets suggest that these dietary carhohydrate sources are poorly utilized for energy purposes In contrast, in chinook salmon, poor growth, decreased liver weight, liver glycogen and carcass fat content were reported in fish fed with higher molecular weight carbohydrate sources (dextrin or potato starch) than with simple sugars (Buhler and Halver, 1961)

The lowest growth and poor feed efficiency observed in *H. fossilis* with α-cellulose containing diet is understandable since this nutrient does not contribute any calories, and in the absence of adequate non-protein energy source in the diet, protein energy seems to get utilized to fulfil energy demands of the fish

In conclusion, results on omnivorous *H. fossilis* clearly indicate that dietary monosaccharides (glucose, fructose) and disaccharide (maltose), and α-cellulose are poorly utilized by this fish in comparison to sources like dextrin, pre-cooked corn-starch or sucrose

SUMMARY

Growth, conversion efficiency, body composition, nutrient retention and plasma glucose concentration were evaluated in fingerling catfish, *H fossilis* (6 20±1 0 cm, 3 27±0 04 g), fed iso-nitrogenous (40% CP), iso-caloric (4 70 kcal g^{-1}, gross energy) test diets, containing different sources of carbohydrate (glucose, fructose, maltose, sucrose, dextrin, pre-cooked corn - starch or α- cellulose) at 20% level of inclusion Each dietary treatment had three replicates of twenty fish each The growth trial, conducted in 70 l high density polyvinyl flow-through (1-1 5 l/min) type indoor circular troughs (water volume 55 l) lasted for six weeks Fish were fed to apparent satiation, six days a week, twice daily at 0800 and 1600 h Maximum growth and best feed conversion efficiencies (*P 0.05*) were obtained with dextrin containing diet Growth and conversion efficiencies were minimum in fish fed α-cellulose based diet Variations in plasma glucose were significant (*P 0 05*)

with respect to level (maximum and minimum) and recovery over the 8 hour sampling time Post-feeding glucose or maltose resulted in maximum increase in plasma glucose, followed by post-feeding sucrose, fructose, or dextrin containing diets Post-feeding pre-cooked corn-starch or α-cellulose produced relatively low (*P 0.05*) variation in plasma glucose level All experimental groups at the end of the study exhibited significantly (*P 0.05*) higher percentages of crude lipid, ash and body energy, and lower percentages of body moisture Maximum protein retention in fish was noted with dextrin containing diet, while higher fat and energy retention was seen with sucrose based diet Dietary α-cellulose produced significantly (*P 0.05*) lower value for protein, fat and energy retention The results indicated that, although the fish was able to utilize different dietary carbohydrate sources, the efficacy of their utilization was affected by the nature/complexity of the carbohydrate fed

Table 1. Ingredient and proximate composition of experimental diets.

Ingredients, g/100 g diet (as fed basis)	Amount (%)
Casein (84% CP)	38.09
Gelatin (87.6% CP)	9.13
Carbohydrates[1]	20.00
Corn oil	4.70
Codliver oil	2.35
Vitamin premix[2]	1.00
Mineral premix[2]	4.00
Carboxymethyl cellulose	5.00
α-cellulose	15.73
Nutrients (% dry matter)[3]	
Crude protein	40.00
Crude fat	7.10
Crude ash	10.00
Nitrogen-free extract[4]	20.00
Crude fibre	22.90
Gross energy ($kcal.g^{-1}$)[5]	4.70

[1] Carbohydrates: either glucose, fructose, maltose, sucrose, dextrin, pre-cooked corn-starch or α-cellulose.

[2] Halver (1976)

[3] Mean of 3-5 determinations.

[4] By difference

[5] Based on determined values on ballistic bomb calorimeter.

Table 2. Growth, feed conversion efficiencies and survival in *H. fossilis* fed different sources of dietary carbohydrate*

Diets	Mean initial body weight (g)	Mean final body weight (g)	Weight gain (%)	Specific growth rate (%)	Feed intake (mg/fish day^{-1})	Feed conversion ratio	Protein efficiency ratio	Survival (%)
Glucose	3.37±0.10	$7.90±0.64^{d}$	$131.98±7.35^{c}$	$2.03±0.15^{d}$	$189.29±5.11^{c}$	$1.74±0.16^{b}$	$1.44±0.13^{d}$	97.00
Fructose	3.13±0.12	$6.50±0.72^{b}$	$107.40±6.97^{b}$	$1.74±0.13^{b}$	$157.14±4.20^{a}$	$1.96±0.04^{c}$	$1.27±0.13^{b}$	97.00
Maltose	3.35±0.11	$7.05±0.92^{c}$	$109.00±9.74^{b}$	$1.81±0.14^{c}$	$166.67±5.14^{b}$	$1.88±0.11^{c}$	$1.33±0.16^{c}$	98.00
Sucrose	3.22±0.14	$8.23±0.98^{e}$	$155.70±9.42^{d}$	$2.24±0.11^{e}$	$180.95±6.10^{c}$	$1.52±0.12^{a}$	$1.65±0.12^{e}$	99.00
Dextrin	3.20±0.14	$8.52±0.71^{f}$	$166.15±11.30^{e}$	$2.30±0.14^{ef}$	$190.48±10.11^{c}$	$1.50±0.16^{a}$	$1.67±0.16^{e}$	100.00
Corn-starch	3.27±0.15	$8.19±0.66^{e}$	$150.40±11.95^{d}$	$2.19±0.12^{e}$	$198.81±7.42^{c}$	$1.70±0.11^{b}$	$1.47±0.19^{d}$	100.00
α–cellulose	3.35±0.14	$6.19±0.66^{a}$	$84.66±11.77^{a}$	$1.45±0.12^{a}$	$221.90±17.41^{d}$	$3.28±0.17^{d}$	$0.77±0.12^{a}$	99.00

* Results are mean of triplicate runs ± SEM. N=60 fish each/dietary treatment. Values in the same column not sharing the same superscripts are significantly (P<0.05) different.

Table 3. Body composition and nutrient retention effeciencies in *H. fossilis* fed different sources of dietary carbohydrate*

Diets	Body composition (% wet weight)					Nutrient retention efficiency (%)		
	Moisture[1]	Crude protein	Crude fat	Ash	Energy[1] ($kcal.g^{-1}$)	Protein	Fat	Energy
Initial fish[2]	78.21 ± 0.63^{g}	13.92 ± 0.11^{b}	2.21 ± 0.01^{a}	3.04 ± 0.09^{c}	5.06 ± 0.09^{a}	—	—	—
Glucose	73.25 ± 0.22^{d}	15.10 ± 0.08^{cd}	4.96 ± 0.09^{d}	2.5 ± 0.02^{a}	5.55 ± 0.04^{c}	22.71 ± 6.74^{c}	56.34 ± 2.47^{c}	74.85 ± 8.53^{c}
Fructose	74.52 ± 0.28^{e}	14.96 ± 0.11^{c}	5.08 ± 0.11^{d}	2.54 ± 0.07^{a}	5.67 ± 0.02^{d}	0.39 ± 6.43^{b}	55.77 ± 2.43^{c}	71.75 ± 7.72^{c}
Maltose	71.13 ± 0.50^{c}	14.74 ± 0.17^{c}	4.32 ± 0.17^{c}	2.95 ± 0.04^{c}	5.22 ± 0.01^{b}	20.82 ± 6.24^{b}	46.28 ± 2.59^{b}	63.86 ± 9.78^{b}
Sucrose	68.08 ± 0.24^{a}	15.75 ± 0.08^{e}	8.31 ± 0.18^{g}	2.71 ± 0.04^{ab}	5.92 ± 0.02^{e}	28.15 ± 9.41^{d}	114.61 ± 2.95^{e}	94.50 ± 8.58^{f}
Dextrin	69.32 ± 0.23^{b}	17.40 ± 0.07^{g}	6.78 ± 0.08^{e}	2.98 ± 0.02^{d}	5.76 ± 0.02^{e}	33.11 ± 9.27^{e}	89.98 ± 1.20^{d}	90.23 ± 9.55^{e}
Corn-starch	69.89 ± 0.41^{b}	17.13 ± 0.13^{f}	7.77 ± 0.27^{f}	2.65 ± 0.04^{a}	6.01 ± 0.08^{e}	28.81 ± 6.40^{d}	95.39 ± 4.19^{d}	85.97 ± 8.34^{d}
α-cellulose	76.50 ± 0.46^{f}	12.67 ± 0.08^{a}	3.64 ± 0.22^{b}	2.59 ± 0.04^{a}	5.28 ± 0.08^{b}	8.67 ± 9.21^{a}	23.28 ± 2.31^{a}	38.89 ± 11.62^{a}

* Results are mean of triplicate runs ± SEM. N=5 fish each/dietary treatment. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.

[1] Dry matter basis

[2] N=10 fish.

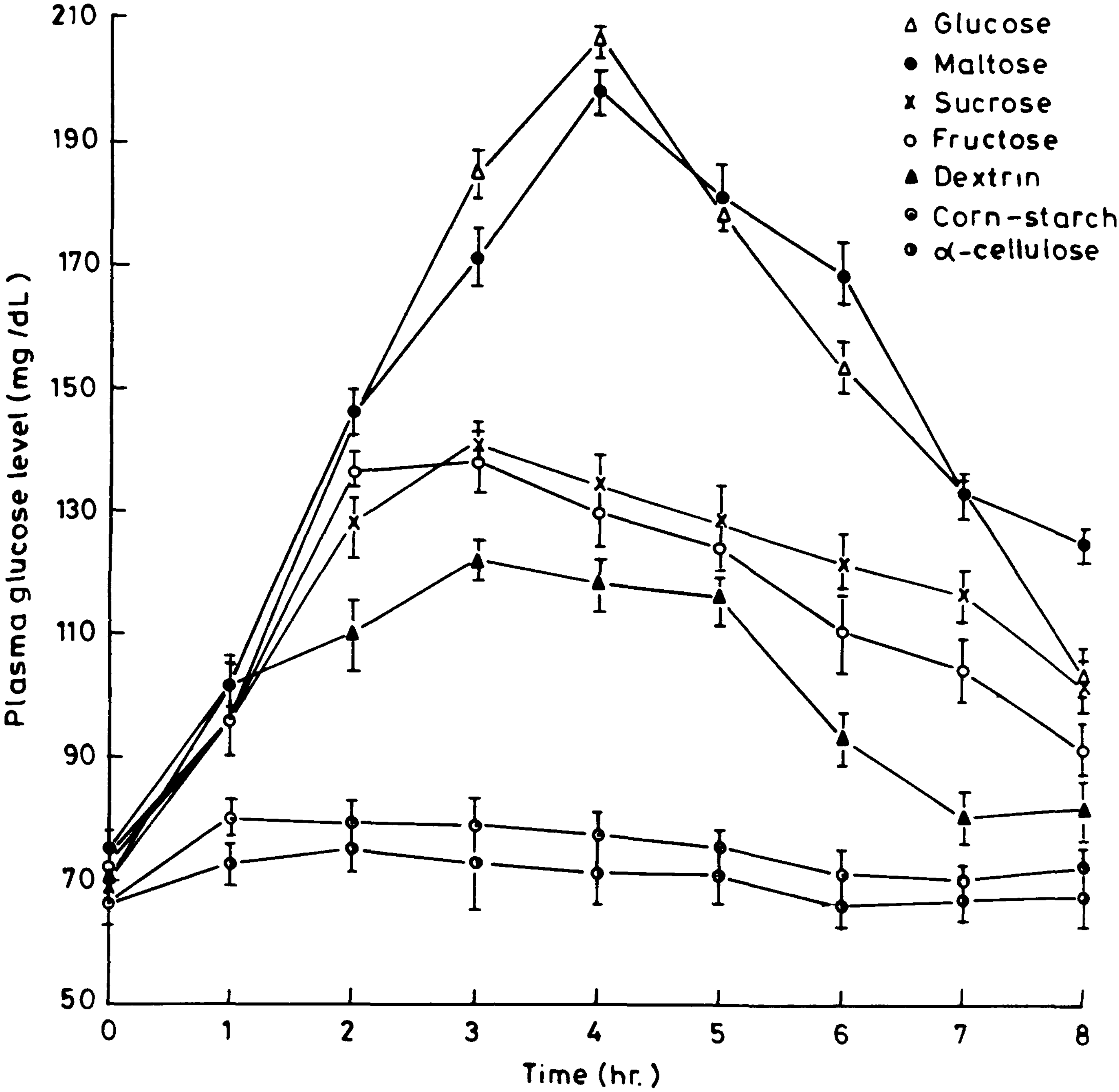


Fig. 1. Plasma glucose level of *H. fossilis* fed different carbohydrate sources. Each point represents mean±SEM (N=4).

Chapter III

CHAPTER III

EFFECTS OF VARYING DIETARY CARBOHYDRATE-TO-LIPID RATIO ON THE GROWTH, FEED CONVERSION, NUTRIENT RETENTION, AND BODY COMPOSITION OF INDIAN MAJOR CARPS AND THE WALKING CATFISH

INTRODUCTION

In a successful fish husbandry practice, consideration is generally given to dietary protein component to produce optimal fish growth Equally important is the inclusion of appropriate levels of non-protein energy sources in the diet that determine the efficiency of protein utilization (Steffens, 1981 , and Wilson and Halver, 1986) Carbohydrate and lipid are the major non-protein energy sources in fish diet Compared to dietary lipid, carbohydrates are relatively inexpensive and more readily available source of energy to many fish species In warmwater fish, dietary carbohydrate utilization is considerably high, and incorporation of this nutrient has added beneficial effects on pelleting quality of the diet and fish growth (NAS - NRC,1983, Lovell, 1989 , and Hidalgo *et al.*, 1993) Increased levels of dietary lipid inclusion, besides creating problems like poor pelleting and development of rancidity in stored feed (Jauncey, 1982), may reduce feed intake and assimilation, preventing the intake of necessary amounts of protein and other nutrients for maximum fish growth It may also adversely affect fish carcass/body composition (Takeuchi *et al* ,1978[a,b] ,Millikin, 1983, Piper *et al.*,1989, and Hanley, 1991)

Since fish eat to satisfy their body energy needs, any imbalance with respect to non-protein energy sources and/or their levels of inclusion may have a direct bearing on growth, conversion efficiencies, nutrient retention, and body composition It is thus imperative to work out, for each fish species, optimum dietary carbohydrate-to-lipid ratio (CHO L) that produces the best growth, feed conversion, and improved nutrient retention/body composition Earlier studies on these aspects were mostly confined to channel catfish (Garling and Wilson, 1977), tilapia (El-Sayed and Garling, 1988), hybrid striped bass (Nematipour *et al.*, 1992), and hybrid *Clarias* catfish (Jantrarotai *et al* , 1994), with almost no information on Indian cultivable finfish species

In the inland fisheries sector, Indian major carps, namely,*C catla, L rohita* and *C mrigala,* comprise the major capture/culture fisheries (Jhingran, 1991) These carps are fast growing, attaining marketable size of 800 - 1000 g in less than a year, and are generally propagated on extensive or semi-intensive lines in polyculture system (Jhingran and Pullin, 1988) Previous studies on the nutrition of these fishes remained confined to their protein and amino acid requirements (Sen *et al.,* 1978 , Singh and Bhanot, 1988 , De Silva and Gunasekera, 1991 , Ravi and Devaraj, 1991 , and Khan and Jafri, 1991[a] , 1993)

C. batrachus, the walking catfish, is widely distributed throughout the Indian sub-continent Due to its fast growth and high marketability, it is mostly cultured either alone or in conjunction with other catfish species on extensive lines (Thakur, 1991) Lack of studies on its nutrition remains a major impediment in the development of intensive culture of this important catfish Information on the nutrition of this species seems limited to its protein and lipid (AICRP, 1987 , Chuapoehk, 1987 , Khan and Jafri, 1990, 1991[b], and

Anwar and Jafri, 1995), and energy and protein maintenance (Hassan and Jafri, 1994) requirements

The present work deals with the effects of varying CHO L ratios on the growth, feed conversion, nutrient retention, and body composition, of the three species of Indian major carps and the walking catfish

MATERIALS AND METHODS

Experimental diets

Casein-gelatin based iso-nitrogenous (40% CP) and iso-caloric (3 46 kcal g^{1},ME) semi-purified diets were formulated (Table 1) A mixture of corn and cod liver oil (2 1) was used as dietary lipid Dextrin was used as carbohydrate source The vitamin and mineral premixes were according to Halver (1976) Metabolizable energy (ME) in the diet was calculated using physiological fuel values as quoted elsewhere (page 19) Crude protein in the experimental diets was fixed according to the requirement of the concerned species (Khan and Jafri, 1990 , and 1991[a]) The diets differed only in their CHO L ratio (Table 1), achieved at a rate of 2 25 1, commensurate with carbohydrate-to-lipid physiological (ME) values (Garling and Wilson, 1977 ,and El-Sayed and Garling, 1988), and ranged from 0 02 to 43 00 (g g) The preparation of the experimental diet has been described under General Methodology (page 14) Proximate composition of diet was determined according to standard methods (page 16 - 19)

Feeding trial

Source of fish, their acclimation, and general experimental design have been described elsewhere (page 12, 15)

Desired number of *C. catla* (2 1 ± 0 5 cm, 0 20 ± 0 01 g), *L. rohita* (2 1 ± 0 4 cm , 0 17 ± 0 01 g), *C. mrigala* (2 2 ± 0 8 cm , 0 31 ± 0 01 g), and *C batrachus* (12 5 ± 1 2 cm , 13 04 ± 0 11 g) were sorted out from the acclimated fish lot and randomly stocked, in triplicate groups, at 30 fish each for the first three and 12 fish each for the last species, in 70 l high density polyvinyl flow-through (1 - 1 5 l/min) type indoor circular troughs (water volume 55 l)

Fishes were fed the experimental diets in the form of moist cake, to apparent satiation, twice daily at 0800 and 1600 h, six days a week over a 6-week period Initial and subsequent weekly weight gains (g) were recorded, after anaesthetizing the fishes with MS 222 solution (1 10,000) Water temperature and dissolved oxygen, over the experimental period, were 29 ± 1°C and 6 8 ± 0 2 ppm, respectively

Growth parameters and feed utilization efficiencies were measured using standard definitions (page 21 - 22)

Proximate composition and gross energy analysis

Before commencement of the feeding trial, 20 - 25 specimens of each carp species, and 10 specimens of the catfish were randomly sacrificed with an overdose of MS 222 solution Pooled samples, in triplicate, were taken for the determination of initial wholebody composition, using standard methods described earlier (page 16- 19) At the end of the feeding trial, twenty fish from each dietary treatment in the case of carps, and five fish from each treatment in the case of catfish were randomly selected and sampled as above for their final body composition Wholebody energy density (kcal g^{-1}) was determined on ballistic bomb calorimeter (page 19)

Statistical analysis

Comparisons among treatment means or between initial or final values of the same treatment were made by one way analysis of variance (*ANOVA*) and Duncan's multiple range test at 0 05% probability level Second degree polynomial regression analysis was employed to weight gain (%) data to predict maximum gain in response to CHO L ratio Simple regression and correlation coefficient (r) were also calculated to establish the relationship between dietary nutrient intake (X) and growth/body constituent (Y)

RESULTS

Growth in fish receiving varying CHO L ratio (0 02 to 43 00) in their diets differed significantly *(ANOVA P 0.05),* producing a quadratic growth pattern (Table 2 - 5) Among the various species, highest weight gain percent and SGR(%) were observed in *C. mrigala* and *C. batrachus* fed 8% lipid and 27% dietary carbohydrate (dry weight basis), corresponding to CHO L ratio of 3 38 *C catla* and *L. rohita*, on the other hand, showed the maximum weight gain and SGR at 4% lipid and 36% dietary carbohydrate, corresponding to CHO L ratio of 8 93 In all the species, fish fed either the lowest (0 02) or the highest (43 00) CHO L ratio tended to produce significantly lower (*P*< 0 05) growth and feed conversion efficiencies The relationship between weight gain and CHO L ratio, depicted through second degree polynomial regression curve, indicates that highest gains in weight would occur in fish at 8 93 CHO L ratio, these being 564%, 466%, 344% and 131% for *C. mrigala, L rohita, C catla,* and *C. batrachus*, respectively (Fig 1 - 4)

The mean FCR and PER differed significantly ($P < 0\ 05$) among the various dietary treatments FCR and PER improved with dietary CHO L ratio, registering the maximum at CHO L ratio of 3 38 in *C. mrigala* (Table 4) and *C. batrachus* (Table 5), and at 8 93 in *C. catla* (Table 2) and *L. rohita* (Table 3) No specific pattern of mortality was noticed among the treatments with respect to CHO L ratio in the diet, the average survival being over 92 %

The body composition and nutrient retention efficiency of fish fed the experimental diets is given in Table 6 - 9 At the end of the study, all experimental groups exhibited higher percentages of crude protein, lipid and body energy, and lower percentages of moisture and ash The relationship between carbohydrate intake (0 44 to 43 00%) and body moisture/ash was positive, while crude protein, lipid and body energy values correlated negatively with carbohydrate intake (Table 10)

Highest protein and energy retention efficiencies were noted in fish having maximum weight gain and body crude protein Fat retention efficiency increased progressively with dietary CHO L ratio (Table 6 - 9)

DISCUSSION

The results of the present study clearly indicate that growth and conversion efficiencies in the three species of the Indian major carps and the walking catfish get affected with the nature and/or levels of non-protein energy source in the diet Reduction in dietary lipid content from 19 95% to 8 07%, with concomitant increase in carbohydrate level from 0 44 to 27 28%, corresponding to CHO L ratios of 0 02 to 3 38, significantly improved the growth as well as conversion efficiencies in *C. mrigala* and *C. batrachus* While in *C. catla* and *L. rohita*, reduction in dietary lipid from 19 95 to 4 08%, with step-wise increase in carbohydrate from 0 44 to 36 42%, corresponding to CHO L ratios of 0 02 to 8 93, improved the above parameters significantly However, in channel catfish, *Ictalurus punctatus*, diet containing 24% CP and 275 kcal/100 g (ME), with CHO L ratios ranging from 0 45 to 4 5, has been reported to produce fish with no significant difference in weight gains, feed conversion, protein and energy deposition/retention (Garling and Wilson, 1977), and in tilapia, *Tilapia zilli*, insignificant effects on growth and performance were noted in fish fed CHO L ratios ranging from 0 81 to 8 76 in a 30% CP and 300 kcal/100 g (ME) diet (El-Sayed and Garling, 1988) Similarly, in striped bass (Nematipour *et al.*, 1992) and rainbow trout (Brauge *et al.*, 1993), feeding different CHO L ratio diets did not affect SGR, feed gain ratio and protein retention More recently, in hybrid *Clarias* catfish (*Clarias macrocephalus X C. gariepinus*) feeding different CHO L ratios did not produce significant difference in weight gain, feed efficiency and protein efficiency ratio, and protein retention (Jantrarotai *et al* , 1994) In the present experiment, fishes were fed the required levels of dietary protein, optimum E/P ratio (Anonymous, 1991, Hassan and Jafri, 1994), and other essential micronutrients (Halver, 1976), and diets differed only in the nature of non-protein energy supplying nutrients (CHO L) The differences observed in the growth and feed efficiencies indicate the varying ability of the Indian major carps and the walking catfish to adapt to increasing levels of dietary carbohydrate which appeared relatively more pronounced in these fishes than that reported for channel catfish and tilapia (Garling and Wilson, 1977 , and El-Sayed and Garling, 1988) The beneficial effects of dietary carbohydrate in warmwater fish is well documented (NAS - NRC, 1983 , Anderson *et al.*, 1984 , Furuichi *et al.*, 1986 , Mollah and Alam, 1990 , and Erfanullah and Jafri, 1993 , 1994) In other fish species, feeding varying levels of non-protein energy at identical protein and energy levels is reported to affect fish growth and performance In plaice, weight gain of fish was greater in fish fed diets containing both carbohydrate and lipid, than those receiving lipid as the sole dietary non-protein energy source (Cowey *et al.*, 1975) In chinook salmon, growth and efficiency of protein utilization decreased when dietary fat was exchanged isocalorically with carbohydrate (Buhler and Halver, 1961) In yellowtail, and Nile tilapia increased growth and feed utilization was observed on feeding diets containing apppropriate levels of carbohydrate, fat and protein (Shimeno *et al.*, 1985 , and Teshima and Kanazawa, 1986) Compared to trout, carps are known to effectively utilize both dietary carbohydrate and lipid (Dabrowski, 1986)

Reduced growth and poor conversion efficiencies in fish fed diet containing either high lipid with low carbohydrate or low lipid with high carbohydrate have also been reported in chinook salmon (Buhler and Halver, 1961), channel catfish (Dupree and Sneed, 1966, and Garling and Wilson, 1977), rainbow trout (Edwards *et al* , 1977), red drum (Ellis and Reigh, 1991 , and Serrano *et al.*, 1992), and hybrid *Clarias* catfish (Jantrarotai *et al* , 1994) Reduced growth and conversion efficiencies in fish fed high lipid-low carbohydrate diet could be the result of reduced feed intake by the fish due to its high dietary (lipid) caloric density, preventing the intake of necessary amounts of protein and other nutrients required for maximum growth Low growth and poor conversion efficiences with low lipid-high carbohydrate diet could perhaps be to attributed to availability of deficient amount of essential fatty acids (EFAs) in diet It has been pointed out that carp require about 1% 18 3 n-3 and 1% 18 3 n-6 series fatty acids (Takeuchi and Watanabe, 1977), while omnivorous channel catfish requires about 1% 18 3 n-3 series fatty acids (Stickney *et al*, 1983 , and Robinson and Lovell, 1984) for maximum growth Since diet with CHO L ratio of 0 02 contained only 1% dietary lipid, it would not have matched the needs of the fish for essential fatty acids thus resulting in reduced growth and poor feed efficiency In *T. zilli*, low lipid (1 7%) and high carbohydrate (41 00%), corresponding to CHO L ratio of 24 11, in a 30% CP and 300 kcal/100 g (ME) diet similarly produced low growth rates and feed utilization (El-Sayed and Garling, 1988)

The maximum growth and best feed utilization observed in *C batrachus* fed diet containing 8 07% dietary lipid seems in agreement with the findings on channel catfish (Dupree, 1966 , and Stickney, 1984), rainbow trout (Watanabe *et al*, 1979 , and Reinitz and Hitzel, 1980), red drum (Williams and Robinson, 1988 , Ellis and Reigh, 1991 , and Serrano *et al*, 1992), hybrid *Clarias* catfish (Jantrarotai *et al*, 1994) and tilapia (Hanley, 1991) Utilization of higher dietary carbohydrate levels, in the range of 27 - 36%, noted in Indian major carps also conforms with earlier studies on *L rohita* and *C catla* (Erfanullah and Jafri, 1993, 1994), and the common carp (Takeuchi *et al*, 1979[a] , and Furuichi and Yone, 1980) where dietary carbohydrate in the range of 30 - 38 40% was found to produce higher weight gains and better feed efficiencies

The data obtained on the body composition of different fishes during this study indicate that increased dietary lipid intake influence body composition as was also seen in rainbow trout (Lee and Putnam, 1973 , Takeuchi *et al*, 1978[a,b,c] Reinitz and Hitzel, 1980 , and Brauge *et al*, 1993), channel catfish (Garling and Wilson, 1977), tilapia (El-Sayed and Galing, 1988 , and Hanley, 1991), red drum (Ellis and Reigh, 1991, Santhan and Gatlin, 1991 , and Serrano *et al.*, 1992), hybrid striped bass (Nematipour *et al.*, 1992), hybrid *Clarias* catfish (Jantrarotai *et al* , 1994) and carp (Dabrowski, 1977 , Takeuchi *et al.*, 1978[c] , and Jauncey,1979) The negative correlation between carbohydrate intake (0 44 to 43 00%) and body lipid/energy content (Table 10) appear interesting, since increased carbohydrate failed to produce undesirable fat accumulation in the body Similarly, in turbot fed increasing percentages of non-protein energy, presented as starch, also reduced lipid deposition (Nijhof and Bult, 1994) Although body crude protein in the experimental fishes, on wet weight basis, was maximum at 0 60 to 1 54 CHO L ratio in diet, maximum protein retention values appeared at 3 38 to 8 93 CHO L ratios, coinciding with the highest weight gain and PER A more clear picture of changes in body crude protein can be seen by recalculating body constituent data on dry weight basis, wherein experimental fish receiving 0 60 to 1 54 CHO L ratio possessed significantly (P < 0 05) lower body crude protein than those receiving CHO L ratios in the range of 3 38 to 43 00 (Table 11)

It may thus be summarized, on the basis of weight gain, feed conversion, nutrient retention, and body composition that 27 - 36% carbohydrate and 8 - 4% lipid, corresponding to CHO L ratio of 3 38 and 8 93, in a 40 % CP and 3 46 kcal g^{-1} ME diet, with an E/P ratio of 8 64, is optimal for *C batrachus*, *C. mrigala*, *C. catla* and *L. rohita* However, based on the predicted weight gain, using second degree polynomial regression analysis, it can be stated that maximum weight gain in these fishes could occur at CHO L ratio of 8 93 in diet Higher dietary carbohydrate with low lipid (43 00, CHO L) or lower dietary carbohydrate with high lipid (0 02, CHO L) may result in lower weight gains and poor conversion, and also affect the body composition of fish

SUMMARY

Effects of varying carbohydrate-to-lipid (CHO L) ratios on the growth, conversion efficiencies, body composition, and nutrient retention efficiency were studied in the Indian major carps fry, *C. catla* (2 10±0 5 cm, 0 20±0 01 g), *L rohita* (2 10±0 4 cm, 0 17±0 01 g), and *C mrigala* (2 20±0 8 cm, 0 31 ±0 01 g), and in young walking catfish, *C.batrachus* (12 50± 2 cm, 13 04±0 11 g) Iso-nitrogenous (40% CP) and iso-caloric (3 46 kcal g^{-1}, ME) semi-purified diets, with varying CHO L ratio (0 02, 0 60, 1 54, 3 38, 8 93 or 43 00 g g), were fed to triplicate groups of thirty fish each in the case of carps and twelve fish each in the case of catfish in 70 l high density polyvinyl flow-through (1-1 5 l/min) type indoor circular troughs (water volume 55 l) Fish were fed to apparent satiation, six days a week, twice daily at 0800 and 1600 h Over the 6-week growth trial, growth rates in fishes differed significantly *(P 0 05)* with CHO L ratio in the diets, producing a quadratic pattern Maximum weight gain (%) and SGR(%) were observed in *C mrigala* and *C batrachus* with 8% lipid and 27% carbohydrate diet, corresponding to a CHO L ratio of 3 38 *C. catla* and *L. rohita* showed the maximum weight gain and SGR at 4% lipid and 36% carbohydrate, corresponding to a CHO L ratio of 8 93 In all the species, fish fed either the lowest (0 02) or the highest (43 00) CHO L ratio tended to produce significantly lower *(P 0 05)* growth and conversion efficiencies At the end of the study, all experimental groups

exhibited higher percentages of crude protein, fat and body energy, and lower percentages of moisture and ash Highest protein and energy retention efficiencies were noted in fish with maximum weight gain and body crude protein Fat retention efficiency increased progressively with dietary CHO L ratio The findings suggest optimal carbohydrate and lipid levels, in 40% CP and 3 46 kcal g^{-1} ME diet, to be 36% and 4% respectively, corresponding to CHO L ratio of 8 93, for the fish species investigated

Table 1. Ingredient and proximate composition of experimental diets.

Ingredient g/100 g diet (as fed basis)	Test diets					
Basal premix[1]	55.22	55.22	55.22	55.22	55.22	55.22
White dextrin	0.00	9.00	18.00	27.00	36.00	42.75
Corn oil	13.33	10.67	8.00	5.33	2.67	0.00
Codliver oil	6.67	5.33	4.00	2.67	1.33	1.08
α-cellulose	24.78	19.78	14.78	9.78	4.78	0.95
	Nutrients (% dry weight)[2]					
Crude protein	40.07	40.05	39.96	40.03	40.06	40.01
Crude fat	19.95	16.02	12.04	8.07	4.08	1.00
Ash	7.04	7.09	7.08	7.08	7.10	7.05
Crude fibre	32.50	27.24	22.39	17.54	12.34	8.94
Nitrogen-free extract	0.44	9.60	18.53	27.28	36.42	43.00
Metabolizable energy ($kcal.g^{-1}$)[3]	3.42	3.49	3.47	3.46	3.46	3.44
CHO: Lipid ratio (g/g)	0.02	0.60	1.54	3.38	8.93	43.00
E/P (as ME)	8.54	8.71	8.68	8.64	8.64	8.60

[1] Basal premix: casein, 38.09; gelatin, 9.13; mineral premix, 4.00 (Halver, 1976); vitamin premix, 1.00 (Halver, 1976) and carboxymethyl cellulose, 3.00 (g), respectively.

[2] Based on 3-4 determinations ± SEM

[3] Calculated on the basis of the physiological fuel values ($kcal.\ g^{-1}$): 4,4 and 9 for protein, carbohydrate and fat, respectively (Garling and Wilson, 1977).

Table 2. Growth and feed efficiency in *C. catla* fed varying carbohydrate to lipid ratio diets.*

CHO:lipid ratio in diets	Mean final body weight (g)	Mean weight gain (g)	Increase in body weight (%)	Specific growth rate (%)	Feed intake (mg/fish day^{-1})	Feed conversion ratio	Protein efficiency ratio	Survival (%)
0.02	0.45±0.18^{a}	0.25±0.14^{a}	126.33±11.34^{a}	1.95±0.12^{a}	27.62±3.11^{a}	4.65±0.32^{d}	0.54±0.04^{a}	92.00
0.60	0.53±0.12^{b}	0.33±0.16^{b}	169.67±10.94^{b}	2.36±0.02^{b}	28.33±4.11^{ab}	3.60±0.08^{c}	0.69±0.02^{b}	94.00
1.54	0.63±0.13^{c}	0.44±0.17^{c}	224.33±18.63^{c}	2.80±0.14^{c}	31.19±5.12^{c}	2.96±0.08^{b}	0.85±0.02^{c}	94.00
3.38	0.76±0.17^{d}	0.56±0.16^{d}	284.00±9.95^{d}	3.19±0.03^{d}	35.48±3.12^{d}	2.66±0.01^{a}	0.94±0.01^{d}	96.00
8.93	0.82±0.16^{de}	0.63±0.18^{de}	332.00±8.63^{e}	3.55±0.11^{d}	36.19±8.21^{d}	2.41±0.15^{a}	1.07±0.03^{b}	96.00
43.00	0.80±0.19^{d}	0.60±0.17^{d}	299.33±9.60^{d}	3.30±0.06^{de}	35.71±3.31^{d}	2.50±0.06^{a}	1.00±0.03^{e}	96.00

* Results are mean of triplicate runs ± SEM. N = 90 fish each/dietary treatment.
Figures in the same column not sharing the same superscripts are significantly ($P<0.05$) different.

Mean initial body weight (g): 0.20±0.01; 0.20±0.01; 0.20±0.01; 0.20±0.01; 0.19±0.01; 0.20±0.01 in 0.02; 0.60; 1.54; 3.38; 8.93 and 43.00 CHO:lipid ratio diets.

Table 3. Growth and feed efficiency in *L. rohita* fed varying carbohydrate to lipid ratio diets.*

CHO:lipid ratio in diets	Mean final body weight (g)	Mean weight gain (g)	Increase in body weight (%)	Specific growth rate (%)	Feed intake (mg/fish day^{-1})	Feed conversion ratio	Protein efficiency ratio	Survival (%)
0.02	0.58 ± 0.13^{a}	0.39 ± 0.13^{a}	210.67 ± 11.38^{a}	2.70 ± 0.09^{a}	30.48 ± 7.11^{b}	3.28 ± 0.10^{d}	0.76 ± 0.02^{a}	97.00
0.60	0.60 ± 0.12^{a}	0.43 ± 0.12^{a}	259.67 ± 14.72^{b}	3.03 ± 0.01^{b}	29.05 ± 6.14^{b}	2.82 ± 0.13^{c}	0.88 ± 0.04^{b}	98.00
1.54	0.73 ± 0.11^{b}	0.56 ± 0.10^{b}	332.00 ± 13.25^{c}	3.49 ± 0.01^{c}	30.95 ± 2.21^{c}	2.32 ± 0.02^{ab}	1.08 ± 0.02^{c}	96.00
3.38	0.95 ± 0.13^{c}	0.70 ± 0.24^{c}	400.67 ± 17.10^{d}	3.84 ± 0.03^{d}	36.43 ± 7.11^{d}	2.18 ± 0.06^{a}	1.15 ± 0.03^{c}	98.00
8.93	0.95 ± 0.12^{d}	0.78 ± 0.13^{d}	452.33 ± 10.62^{e}	4.07 ± 0.05^{e}	38.10 ± 5.51^{e}	2.04 ± 0.06^{a}	1.23 ± 0.04^{d}	98.00
43.00	0.60 ± 0.12^{a}	0.43 ± 0.12^{a}	251.33 ± 10.33^{b}	2.99 ± 0.07^{b}	27.38 ± 2.21^{a}	2.67 ± 0.15^{c}	0.94 ± 0.06^{b}	96.00

* Results are mean of triplicate runs ± SEM. $N = 90$ fish each/dietary treatment.
Figures in the same column not sharing the same superscripts are significantly ($P<0.05$) different.

Mean initial body weight (g): 0.19±0.01; 0.17±0.01; 0.17±0.01; 0.17±0.01; 0.17±0.01; 0.17±0.01 in 0.02; 0.60; 1.54; 3.38; 8.93 and 43.00 CHO:lipid ratio diets.

Table 4. Growth and feed efficiency in *C. mrigala* fed varying carbohydrate to lipid ratio diets.*

CHO:lipid ratio in diets	Mean final body weight (g)	Mean weight gain (g)	Increase in body weight (%)	Specific growth rate (%)	Feed intake (mg/fish day^{-1})	Feed conversion ratio	Protein efficiency ratio	Survival (%)
0.02	1.23±0.12^{a}	0.93±0.18^{a}	310.00±12.45^{a}	3.36±0.05^{a}	57.86±7.11^{a}	2.61±0.08^{c}	0.96±0.03^{b}	96.00
0.60	1.81±0.16^{c}	1.51±0.16^{b}	502.00±17.33^{b}	4.27±0.07^{b}	79.29±7.23^{c}	2.20±0.06^{b}	1.14±0.03^{c}	94.00
1.54	1.99±0.13^{cd}	1.69±0.12^{c}	547.00±15.34^{bc}	4.45±0.02^{bc}	80.00±7.14^{c}	1.99±0.02^{a}	1.27±0.03^{d}	96.00
3.38	2.10±0.16^{d}	1.79±0.16^{d}	579.00±21.59^{c}	4.56±0.07^{c}	82.86±8.42^{c}	1.94±0.05^{a}	1.28±0.03^{d}	96.00
8.93	1.90±0.18^{c}	1.59±0.12^{b}	513.00±10.50^{b}	4.32±0.04^{b}	86.43±7.61^{d}	2.28±0.03^{b}	1.09±0.01^{c}	98.00
43.00	1.34±0.14^{b}	1.03±0.14^{a}	329.00±12.05^{a}	3.46±0.07^{a}	70.71±5.61^{b}	2.88±0.08^{d}	0.87±0.02^{a}	96.00

* Results are mean of triplicate runs ± SEM. $N = 90$ fish each/dietary treatment.
Figures in the same column not sharing the same superscripts are significantly ($P<0.05$) different.

Mean initial body weight (g): 0.30±0.01; 0.30±0.01; 0.31±0.01; 0.31±0.01; 0.31±0.01; 0.31±0.01 in 0.02; 0.60; 1.54; 3.38; 8.93 and 43.00 CHO:lipid ratio diets.

Table 5. Growth and feed efficiency in *C. batrachus* fed varying carbohydrate to lipid ratio diets.*

CHO:lipid ratio in diets	Mean final body weight (g)	Mean weight gain (g)	Increase in body weight (%)	Specific growth rate (%)	Feed intake (mg/fish day^{-1})	Feed conversion ratio	Protein efficiency ratio	Survival (%)
0.02	19.67±1.16^{a}	6.64±6.09^{a}	51.00±1.76^{a}	0.98±0.01^{a}	555.95±7.11^{a}	3.52±0.04^{f}	0.71±0.01^{a}	96.0
0.60	23.86±1.08^{b}	10.82±3.08^{b}	83.00±2.73^{b}	1.44±0.01^{b}	573.81±2.14^{b}	2.22±0.01^{e}	1.12±0.00^{b}	98.00
1.54	30.89±1.10^{e}	17.83±14.11^{e}	136.50±2.15^{e}	2.05±0.01^{e}	602.38±7.91^{c}	1.41±0.01^{b}	1.79±0.01^{e}	100.00
3.38	33.81±1.14^{f}	20.74±3.14^{f}	158.79±13.07^{f}	2.26±0.01^{f}	616.67±8.14^{c}	1.24±0.01^{a}	2.02±0.03^{f}	100.00
8.93	27.30±1.28^{d}	19.28±3.29^{d}	109.76±2.38^{d}	1.76±0.03^{d}	727.38±3.14^{d}	1.58±0.01^{c}	1.58±0.01^{d}	98.00
43.00	24.84±1.45^{c}	11.79±2.45^{c}	90.31±3.32^{c}	1.53±0.04^{c}	583.33±5.61^{b}	2.08±0.04^{d}	1.20±0.03^{c}	96.00

* Results are mean of triplicate runs ± SEM. N = 36 fish each/dietary treatment.
Figures in the same column not sharing the same superscripts are significantly (P<0.05) different.

Mean initial body weight (g): 13.03±0.03; 13.04±0.04; 13.06±0.03; 13.06±0.031; 13.01±0.02; 13.05±0.01 in 0.02; 0.60; 1.54; 3.38; 8.93 and 43.00 CHO:lipid ratio diets.

Table 6. Body composition and nutrient retention efficiencies of *C. catla* fed different carbohydrate to lipid ratio diets*.

Body composition (% wet weight)	Initial** fish	CHO:lipid ratio diets 0.02	0.06	1.54	3.38	8.93	43.00
Moisture***	82.89 ± 0.21^{g}	77.90 ± 0.14^{a}	78.32 ± 0.14^{b}	79.30 ± 0.15^{bc}	80.15 ± 0.16^{c}	80.87 ± 0.18^{c}	81.42 ± 0.16^{d}
Crude protein	11.83 ± 0.10^{a}	12.63 ± 0.06^{ab}	12.83 ± 0.09^{c}	12.65 ± 0.05^{bc}	12.40 ± 0.07^{ab}	12.09 ± 0.07^{a}	11.97 ± 0.07^{a}
Crude fat	0.70 ± 0.09^{a}	6.43 ± 0.06^{g}	5.87 ± 0.08^{f}	5.44 ± 0.07^{e}	4.59 ± 0.09^{d}	3.72 ± 0.05^{c}	2.46 ± 0.09^{b}
Ash	1.99 ± 0.06^{a}	2.03 ± 0.05^{b}	2.19 ± 0.07^{c}	2.23 ± 0.09^{d}	2.16 ± 0.09^{c}	2.14 ± 0.06^{c}	2.19 ± 0.07^{cd}
Energy (kcal.g^{-1})***	4.90 ± 0.04^{a}	6.19 ± 0.04^{f}	6.08 ± 0.05^{e}	6.05 ± 0.07^{e}	5.89 ± 0.09^{d}	5.68 ± 0.08^{c}	5.33 ± 0.09^{b}
				Nutrient retention efficiency (%)			
protein		16.93 ± 2.60^{a}	19.31 ± 2.25^{b}	27.13 ± 2.33^{c}	31.76 ± 2.11^{cd}	38.65 ± 1.60^{e}	32.51 ± 1.55^{d}
Fat		12.25 ± 2.77^{a}	15.48 ± 1.45^{b}	20.75 ± 1.49^{c}	27.64 ± 1.29^{d}	47.21 ± 1.97^{e}	116.43 ± 1.15^{f}
Energy		38.33 ± 3.15^{a}	44.15 ± 1.18^{b}	52.31 ± 1.13^{c}	54.87 ± 3.36^{c}	58.03 ± 3.32^{cd}	51.96 ± 1.15^{c}

* Mean of triplicate runs ± SEM. N = 20 fish each/dietary treatment. Values in the same row not sharing the same superscripts are significantly (P<0.05) different.
** N = 25 fish.
*** Dry matter basis.

Table 7. Body composition and nutrient retention efficiencies of *L. rohita* fed different carbohydrate to lipid ratio diets*.

Body composition (% wet weight)	Initial** fish	CHO:lipid ratio diets 0.02	0.06	1.54	3.38	8.93	43.00
Moisture***	80.22±0.11^{f}	75.53±0.12^{a}	76.33±0.12^{b}	77.63±0.17^{c}	78.56±0.18^{d}	79.55±0.12^{e}	79.77±0.09^{e}
Crude protein	12.70±0.06^{d}	13.25±0.07^{e}	12.75±0.05^{d}	12.48±0.06^{c}	12.11±0.03^{b}	11.95±0.09^{a}	12.10±0.05^{b}
Crude fat	1.81±0.04^{a}	9.10±0.10^{g}	7.59±0.14^{f}	5.95±0.17^{e}	4.13±0.12^{d}	3.49±0.13^{c}	2.66±0.11^{b}
Ash	2.51±0.04^{f}	1.82±0.01^{a}	1.87±0.03^{a}	2.05±0.02^{b}	2.13±0.04^{c}	2.22±0.02^{d}	2.30±0.02^{e}
Energy (kcal.g^{-1})***	5.05±0.02^{a}	6.67±0.02^{g}	6.40±0.02^{f}	6.03±0.01^{e}	5.59±0.02^{d}	5.46±0.01^{c}	5.26±0.04^{b}
				Nutrient retention efficiency (%)			
protein		19.34±1.25^{a}	21.32±1.51^{b}	27.49±1.33^{c}	33.38±1.21^{cd}	34.42±1.61^{cd}	21.06±1.66^{b}
Fat		19.27±1.30^{a}	21.85±1.04^{a}	25.80±1.29^{a}	26.28±1.34^{a}	46.58±1.81^{b}	113.42±9.93^{c}
Energy		54.44±1.63^{b}	57.69±2.67^{b}	64.08±1.47^{c}	63.00±3.35^{c}	66.63±1.14^{c}	47.24±2.61^{a}

* Mean of triplicate runs ± SEM. N = 20 fish each/dietary treatment. Values in the same row not sharing the same superscripts are significantly (P < 0.05) different.

** N = 25 fish.

*** Dry matter basis.

Table 8. Body composition and nutrient retention efficiencies of *C. mrigala* fed different carbohydrate to lipid ratio diets*.

Body composition (% wet weight)	Initial** fish	CHO:lipid ratio diets 0.02	0.06	1.54	3.38	8.93	43.00
Moisture***	80.12 ± 0.13^{f}	74.77 ± 0.18^{a}	75.49 ± 0.13^{b}	76.40 ± 0.14^{c}	77.32 ± 0.20^{d}	78.39 ± 0.12^{e}	78.67 ± 0.15^{e}
Crude protein	12.54 ± 0.05^{a}	13.41 ± 0.06^{c}	13.52 ± 0.05^{c}	13.62 ± 0.12^{cd}	13.34 ± 0.11^{c}	12.83 ± 0.05^{b}	12.81 ± 0.05^{b}
Crude fat	1.72 ± 0.12^{a}	9.83 ± 0.13^{g}	9.09 ± 0.08^{f}	7.48 ± 0.09^{e}	6.49 ± 0.10^{d}	5.62 ± 0.15^{c}	5.33 ± 0.10^{b}
Ash	2.41 ± 0.02^{d}	1.74 ± 0.04^{a}	1.75 ± 0.01^{a}	1.94 ± 0.03^{b}	2.00 ± 0.04^{b}	2.14 ± 0.01^{c}	2.12 ± 0.03^{c}
Energy (kcal.g^{-1})***	5.02 ± 0.04^{a}	6.77 ± 0.04^{g}	6.68 ± 0.03^{f}	6.39 ± 0.04^{e}	6.21 ± 0.05^{d}	6.03 ± 0.06^{c}	5.98 ± 0.07^{b}
			Nutrient retention efficiency (%)				
protein		18.09 ± 2.35^{a}	21.62 ± 1.48^{bc}	27.48 ± 2.44^{e}	29.42 ± 1.32^{e}	24.06 ± 1.26^{d}	20.17 ± 1.35^{b}
Fat		23.85 ± 1.46^{a}	30.13 ± 1.70^{b}	35.66 ± 1.53^{c}	46.71 ± 1.87^{e}	67.76 ± 1.54^{e}	213.09 ± 14.59^{f}
Energy		68.46 ± 1.54^{b}	75.75 ± 2.59^{c}	76.34 ± 1.35^{c}	76.15 ± 2.43^{c}	64.75 ± 1.92^{b}	52.17 ± 2.10^{a}

* Mean of triplicate runs ± SEM. N = 20 fish each/dietary treatment. Values in the same row not sharing the same superscripts are significantly ($P<0.05$) different.
** N = 25 fish.
*** Dry matter basis.

Table 9. Body composition and nutrient retention efficiencies of *C. batrachus* fed different carbohydrate to lipid ratio diets*.

Body composition (% wet weight)	Initial** fish	CHO:lipid ratio diets 0.02	0.06	1.54	3.38	8.93	43.00
Moisture***	79.17 ± 0.15^{g}	73.38 ± 0.18^{a}	74.02 ± 0.10^{b}	75.32 ± 0.12^{c}	75.83 ± 0.18^{d}	76.89 ± 0.15^{e}	77.24 ± 0.19^{f}
Crude protein	14.32 ± 0.06^{a}	15.20 ± 0.12^{e}	15.35 ± 0.05^{f}	14.84 ± 0.05^{bc}	15.04 ± 0.09^{d}	14.73 ± 0.14^{b}	14.59 ± 0.14^{b}
Crude fat	1.91 ± 0.12^{a}	9.01 ± 0.17^{g}	8.09 ± 0.12^{f}	7.06 ± 0.11^{e}	6.36 ± 0.11^{d}	5.69 ± 0.10^{c}	5.00 ± 0.14^{b}
Ash	2.32 ± 0.02^{f}	1.93 ± 0.04^{a}	2.02 ± 0.03^{b}	2.16 ± 0.05^{c}	2.14 ± 0.06^{c}	2.19 ± 0.03^{cd}	2.28 ± 0.07^{e}
Energy (kcal.g^{-1})***	5.20 ± 0.02^{a}	6.54 ± 0.07^{g}	6.40 ± 0.08^{f}	6.24 ± 0.04^{e}	6.15 ± 0.03^{d}	6.06 ± 0.02^{c}	5.89 ± 0.03^{b}
				Nutrient retention efficiency (%)			
protein		17.99 ± 1.25^{a}	19.64 ± 2.20^{b}	30.20 ± 1.23^{d}	34.33 ± 2.44^{e}	23.83 ± 1.30^{c}	17.90 ± 1.17^{a}
Fat		32.60 ± 1.32^{a}	43.56 ± 1.33^{b}	63.54 ± 2.20^{c}	92.01 ± 1.18^{d}	141.69 ± 1.45^{e}	386.80 ± 15.43^{f}
Energy		61.51 ± 2.43^{a}	82.49 ± 2.15^{c}	115.93 ± 1.53^{e}	128.50 ± 1.22^{f}	101.36 ± 1.90^{d}	71.95 ± 4.20^{b}

* Mean of triplicate runs ± SEM. N = 5 fish each/dietary treatment. Values in the same row not sharing the same superscripts are significantly (P<0.05) different.
** N = 10 fish.
*** Dry matter basis.

Table 10. The relationship between dietary carbohydrate (X) and body constituents (Y).

Body composition (% wet weight basis)	Relationship	r	*N*	*P*<
Dietary carbohydrate to				
C. catla				
Moisture*	Y=77.71+0.086X	0.995	18	0.05
Crude protein	Y=12.85-0.019X	-0.902	18	0.05
Fat	Y= 6.76-0.089X	-0.975	18	0.05
Ash	Y= 2.11+0.0019X	0.454	18	n.s.
Energy (kcal. g^{-1})*	Y= 6.28-0.0184X	-0.936	18	0.05
L. rohita				
Moisture*	Y=75.66+0.101X	0.994	18	0.05
Crude protein	Y=13.08-0.0286X	-0.937	18	0.05
Fat	Y= 8.96-0.153X	-0.990	18	0.05
Ash	Y= 1.80+0.0117X	0.990	18	0.05
Energy (kcal. g^{-1})*	Y= 6.67-0.034X	-0.990	18	0.05
C. mrigala				
Moisture*	Y=74.66±0.096X	0.996	18	0.05
Crude protein	Y=13.65-0.017X	-0.809	18	n.s.
Fat	Y= 9.85-0.112X	-0.988	18	0.05
Ash	Y= 1.713+0.0104X	0.967	18	0.05
Energy (kcal. g^{-1})*	Y= 6.79-0.0202X	-0.989	18	0.05
C. batrachus				
Moisture*	Y=73.40+0.098X	0.965	18	0.05
Crude protein	Y=15.26-0.0147X	-0.910	18	0.05
Fat	Y= 8.99-0.170X	-0.997	18	0.05
Ash	Y= 1.95+0.0074X	0.950	18	0.05
Energy (kcal. g^{-1})*	Y= 6.54-0.015X	-0.998	18	0.05

n.s. : not significant.

* Dry matter basis.

Table 11. Body composition of *C. catla*, *L. rohita*, *C. mrigala* and *C. batrachus* fed different CHO:L ratio diets*.

Diets (CHO: L)	Body composition (% dry weight) Dry matter	Crude protein	Crude fat	Crude ash
C. catla				
Initial**	17.11±0.25^{a}	69.15±1.11^{g}	4.08±0.14^{a}	11.66±0.08^{f}
0.02	22.10±0.22^{g}	57.16±1.11^{a}	29.11±0.17^{g}	9.20±0.15^{a}
0.60	21.68±0.17^{f}	59.18±1.13^{b}	27.04±0.15^{f}	10.08±0.15^{b}
1.54	20.70±0.16^{e}	61.14±1.10^{c}	26.29±0.14^{c}	10.77±0.16
3.38	19.85±0.13^{d}	62.44±1.06^{d}	23.11±0.25^{d}	10.89±0.05^{c}
8.93	19.13±0.13^{c}	63.20±1.09^{e}	19.44±0.20^{c}	11.18±0.13^{d}
43.00	18.48±0.19^{b}	64.79±1.09^{f}	13.30±0.13^{b}	11.84±0.15^{e}
L. rohita				
Initial**	19.78±0.28^{a}	64.21±1.13^{g}	9.13±0.17^{a}	12.68±0.08^{g}
0.02	24.47±0.17^{g}	54.16±1.13^{a}	37.20±0.19^{g}	7.46±0.18^{a}
0.60	23.27±0.16^{f}	54.77±1.08^{b}	32.63±0.33^{f}	8.05±0.08^{b}
1.54	22.37±0.15^{e}	55.79±1.08^{c}	26.60±0.22^{e}	9.19±0.11^{c}
3.38	21.44±0.24^{d}	56.47±1.19^{d}	19.25±0.29^{d}	9.92±0.13^{d}
8.93	20.45±0.31^{c}	58.43±1.21^{c}	17.09±0.20^{c}	10.86±0.08^{e}
43.00	20.23±0.22^{b}	59.79±1.44^{f}	13.17±0.10^{b}	11.36±0.08^{f}
C. mrigala				
Initial**	19.88±0.23^{a}	63.07±1.18^{g}	8.66±0.17^{a}	12.16±0.08^{f}
0.02	25.23±0.21^{g}	53.15±1.06^{a}	38.96±0.14^{g}	6.89±0.13^{a}
0.60	24.51±0.27^{f}	55.15±1.10^{b}	37.09±0.13^{f}	7.13±0.07^{b}
1.54	23.60±0.22^{e}	57.72±1.18^{c}	31.69±0.27^{e}	8.21±0.11^{c}
3.38	22.68±0.29^{d}	58.83±1.06^{d}	28.63±0.10^{d}	8.82±0.17^{d}
8.93	21.61±0.26^{c}	59.36±1.09^{e}	26.01±0.09^{c}	9.89±0.09^{e}
43.00	21.33±0.21^{b}	60.09±1.22^{f}	24.97±0.14^{b}	9.96±0.17^{e}
C. batrachus				
Initial**	20.84±0.17^{a}	68.74±1.38^{g}	9.18±0.14^{a}	11.16±0.13^{f}
0.02	26.62±0.24^{g}	57.11±1.06^{a}	33.86±0.18^{g}	7.26±0.17^{a}
0.60	25.98±0.31^{f}	59.10±1.06^{b}	31.12±0.14^{f}	7.79±0.05^{b}
1.54	24.68±0.21^{e}	60.15±1.12^{c}	28.62±0.17^{e}	8.76±0.09^{c}
3.38	24.17±0.18^{d}	62.23±1.08^{d}	26.33±0.13^{d}	8.87±0.12^{c}
8.93	23.11±0.15^{c}	63.78±1.07^{e}	24.63±0.22^{c}	9.49±0.12^{d}
43.00	22.76±0.19^{b}	64.08±1.08^{f}	21.99±0.11^{b}	10.02±0.07^{e}

* Mean of triplicate runs ± SEM $N = 20$ fish each/dietary treatment for *C. catla, L. rohita, C. mrigala;* and $N = 5$ fish each/dietary treatment for *C. batrachus*, respectively.

** $N = 25$ fish for *C. catla, L. rohita, C. mrigala*; and $N = 10$ fish for *C. batrachus.*

Values in the same column for respective fish not sharing the same superscripts are significantly ($P<0.05$) different.

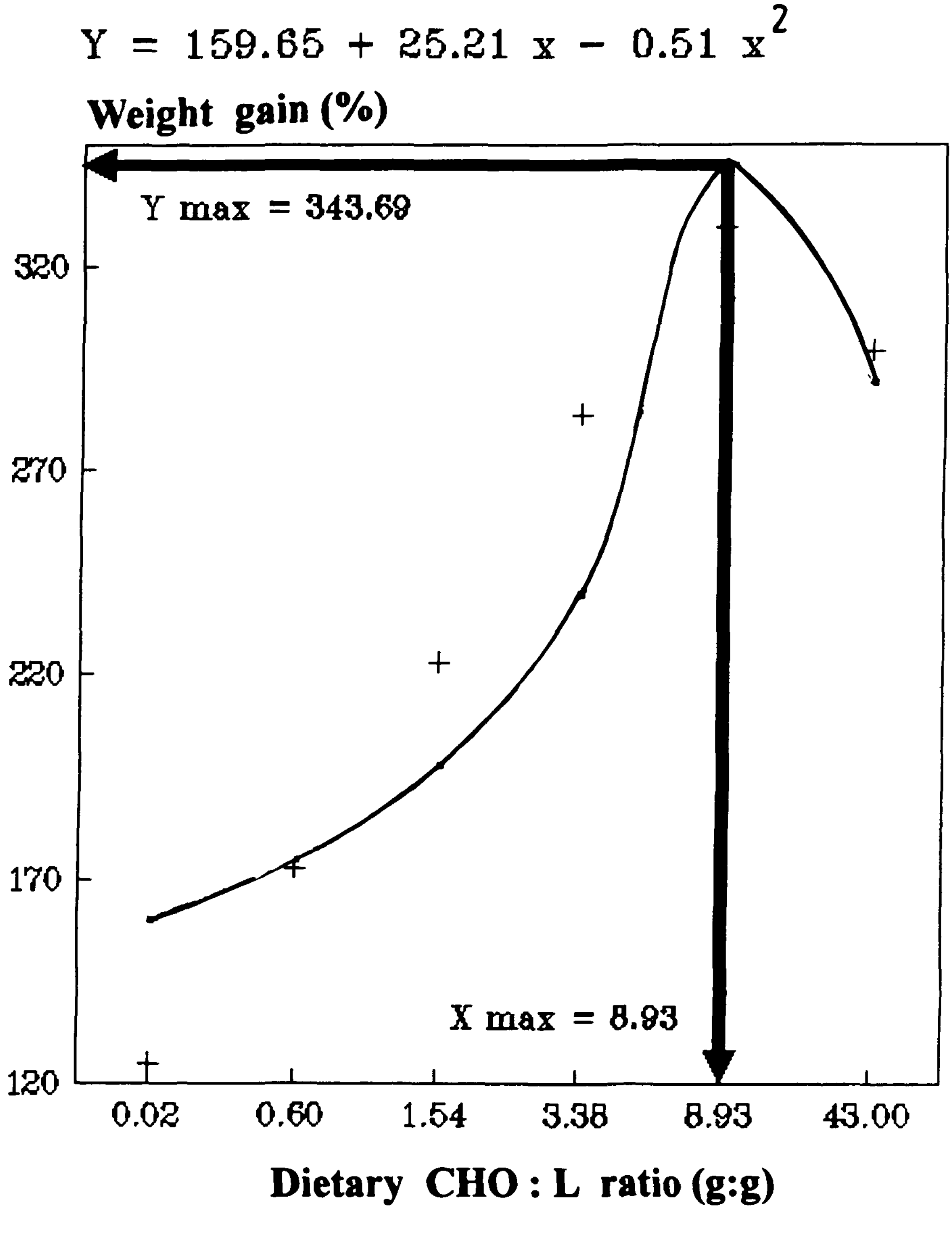


Fig. 1. The second degree polynomial relation of weight gain (%) and dietary CHO:L in *C. catla.*

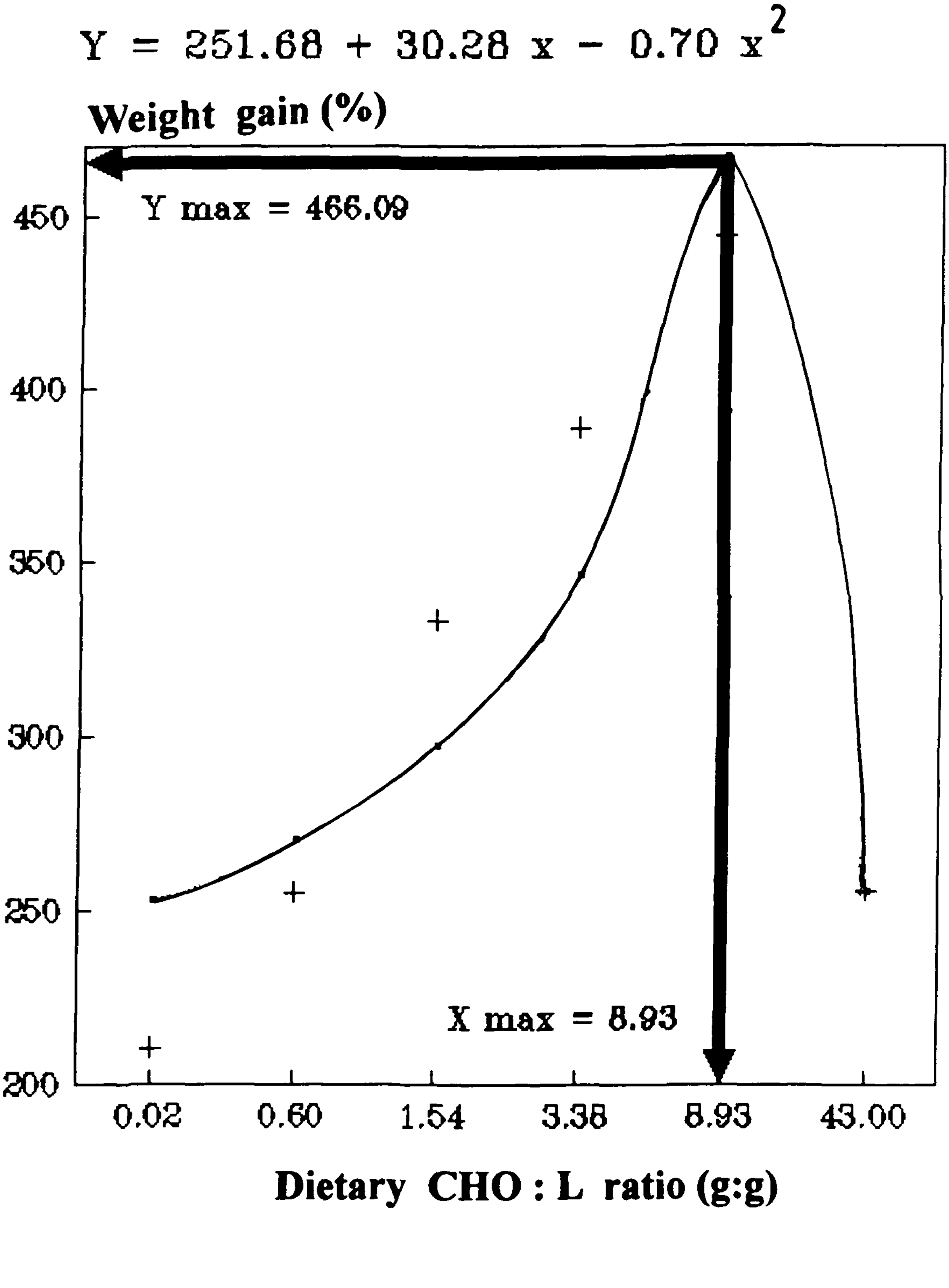


Fig. 2. The second degree polynomial relation of weight gain (%) and dietary CHO:L in *L. rohita*.

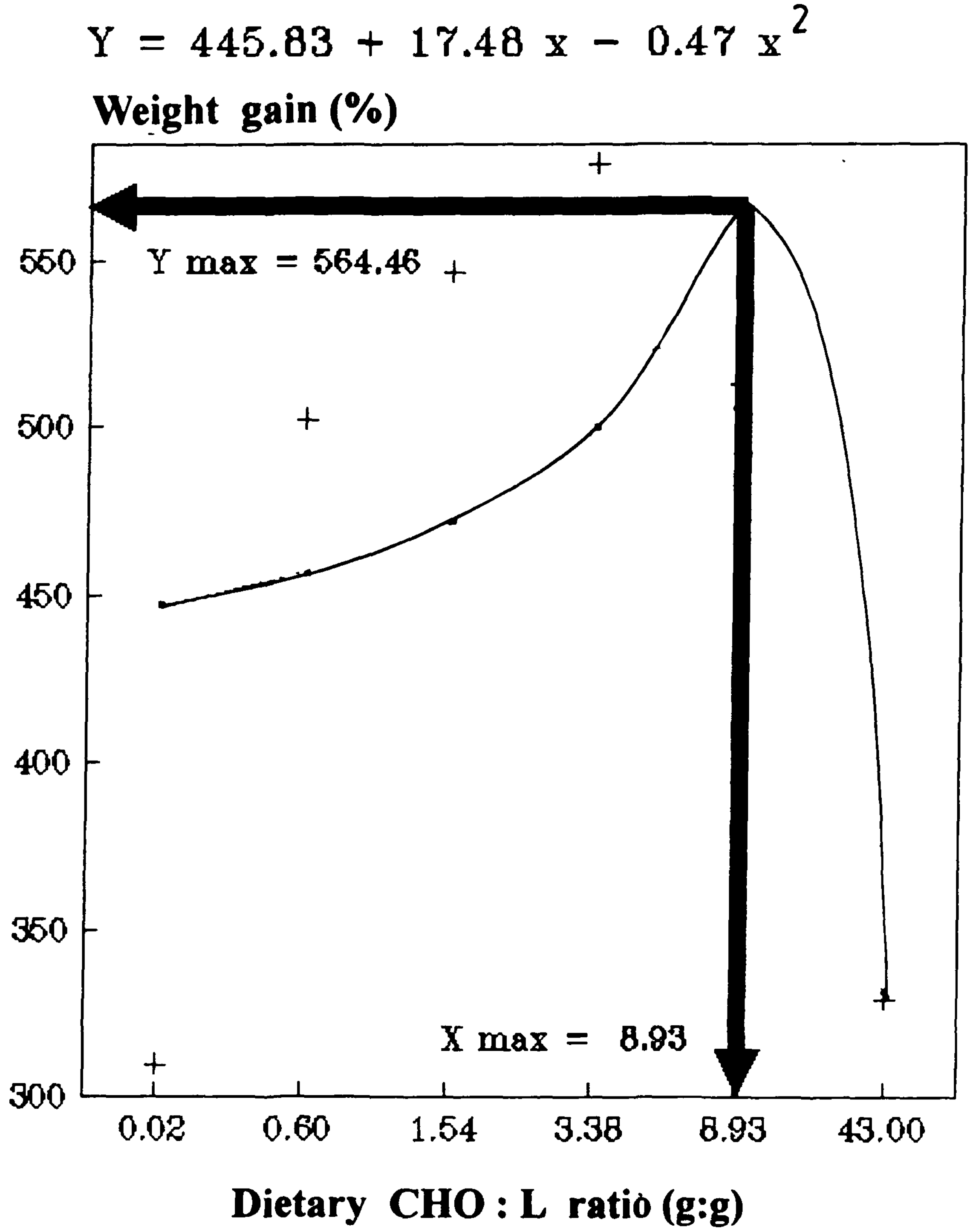


Fig. 3. The second degree polynomial relation of weight gain (%) and dietary CHO:L in *C. mrigala*.

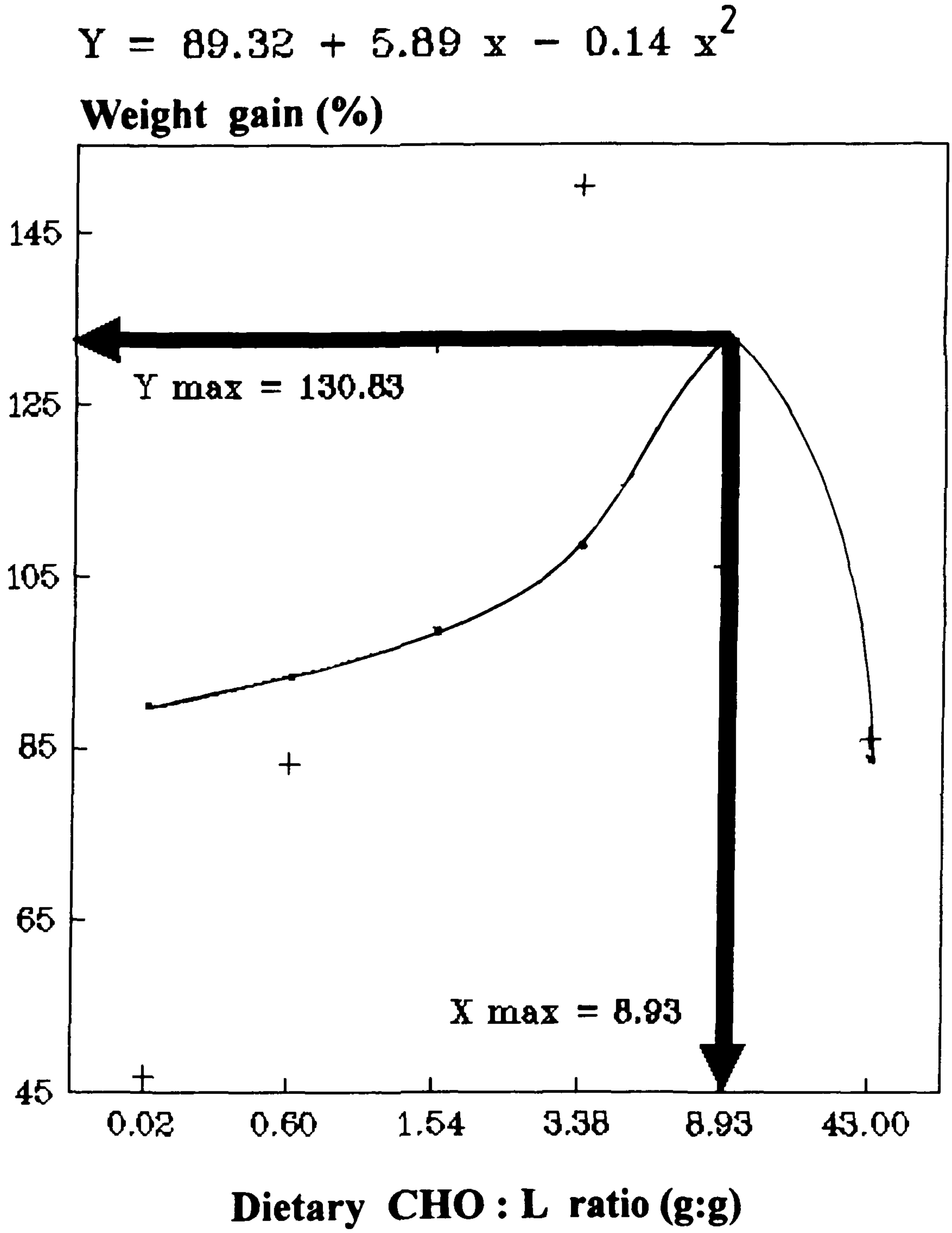


Fig. 4. The second degree polynomial relation of weight gain (%) and dietary CHO:L in *C. batrachus*.

Chapter IV

CHAPTER IV

GROWTH RESPONSE, FEED UTILIZATION AND NUTRIENT RETENTION IN *CATLA CATLA* (HAM.) FRY FED VARYING LEVELS OF DIETARY CARBOHYDRATE

INTRODUCTION

The ability for carbohydrate utilization with respect to its digestibility and metabolism remains unclear in many fish species The level, as also the source and complexity, of carbohydrate seem to affect the availability and utilization of this nutrient in fish (Jauncey, 1982, NAS-NRC, 1983, and Tacon, 1990) Similar to channel catfish, red seabream, European eel and tilapia (Garling and Wilson 1977, Furuichi and Yone, 1980, Anderson, *et al.*, 1984, and Degani and Levanon, 1987), carp are reported to tolerate relatively high levels of carbohydrate in diet (Shimeno 1982 , and Viola and Arieli, 1983) Jauncey (1982) has pointed out that, inspite of higher level of carbohydrate utilization by carp, these fishes show greater disparity in the extent of carbohydrate utilization However, the preference of less costly dietary carbohydrates over protein and fat in feeds may be profitable in many fish species

As already stated, basic nutritional studies on the Indian major carps, including *C. catla*, are relatively few (Sen *et al.*, 1978, De Silva and Gunasekera, 1991, Ravi and Devaraj, 1991, and Khan and Jafri, 1991[a], 1993), and information on carbohydrate nutrition is almost warranted Among the Indian major carps, *C. catla* is the fastest growing fish, attaining marketable size of over 900 g in a year (Jhingran and Pullin, 1988) This study reports the growth response, feed utilization and nutrient retention in *C. catla* fry fed varying levels of dietary carbohydrate

MATERIALS AND METHODS

Experimental diets

Six semi-purified diets (40% CP) were formulated using bread flour as a source of dietary carbohydrate (Table 1) Diets were formulated to provide 30% CP from casein-gelatin mixture and fish meal, and the remaining 10% CP was derived by interchanging defatted soybean meal with bread flour Crude protein content in the experimental diets was fixed according to the requirement of the species (Khan and Jafri, 1991[a]) A mixture of corn and codliver oil (2 1) was used as lipid The vitamin and mineral premixes used were according to Halver (1976) Gross energy (GE) in the diet was quantified by direct calorimetry, while metabolizable energy (ME) was calculated using physiological fuel values (page 19) The GE and ME in the experimental diets ranged from 3 53 - 4 56 and 2 56 - 3 81 kcal g^{-1}, respectively The energy to protein ratio (as ME) in the diets varied from 6 99 to 9 52 kcal g^{-1} protein Details of preparation of experimental diet have been described under General Methodology Section (page 14) Proximate composition of diet was made according to standard methods (page 16 - 19)

Feeding trial

Source of fish, their acclimation, and details of general experimental design have been given elsewhere (page 12, 15)

Hatchery bred *C. catla* (3.0 ± 0.5 cm; 0.28 ± 0.02 g) were sorted out from a previously acclimated fish stock maintained on casein-gelatin based H - 440 semi-purified test diet (Halver, 1976) in wet laboratory, and randomly stocked, in triplicate groups of 30 fish each, in 70 l high density polyvinyl flow-through (1-1.5 l/min) type indoor circular troughs (water volume 55 l). Fish were fed the crumbled experimental diets six days a week, twice daily at 0800 and 1600 h, to apparent satiation. Initial and subsequent weekly weight gains (g) were recorded after anaesthetizing the fish with MS 222 solution (1 10,000) Average water temperature and dissolved oxygen, based on daily measurements, over the experimental period were 28 ± 1°C and 6.7 ± 0 2 ppm, respectively

Growth parameters and feed utilization efficiencies were measured using standard definitions (page 21 - 22).

Proximate composition and gross energy analysis

Before commencement of the feeding trial,35 fish were randomly sacrificed with an overdose of MS 222 solution,and pooled sample,in triplicate, taken for the determination of initial wholebody composition using standard methods (page 16 - 19). At the end of the feeding trial, 12 fish from each dietary treatment were likewise sampled for their final body composition. Wholebody energy was determined on Gallenkamp ballistic bomb calorimeter (page 19).

Statistical analysis

Comparisons among different treatment means or between initial and final values of the same treatment were made by one-way analysis of variance

(*ANOVA*) and Duncan's multiple range test at 0 05% probability level Second degree polynomial regression analysis was employed to SGR(%) data to predict maximum SGR in response to dietary carbohydrate intake Correlation coefficient (r) was calculated to establish the relationship between dietary carbohydrate intake (X) and growth/body constituents/nurtient retention (Y)

RESULTS

Table 2 depicts the growth and feed efficiency of *C. catla* fed different levels of carbohydrate over a six week growth trial The maximum gain in body weight (339%), SGR (3 52%) and PER (1 13), and best FCR (2 35) were observed at 40% dietary carbohydrate intake, beyond which these parameters registered a decline A strong positive relationship (r = 0 99, N = 15, P < 0 05) existed between weight gain (%) and dietary carbohydrate up to 40% intake The relationship between SGR and the level of carbohydrate in diet, depicted through second degree polynomial regression curve, produced a quadratic growth pattern indicating that maximum SGR (3 41%) would occur at 37 69% carbohydrate level, with an E/P ratio of 9 03 kcal g^{-1} protein and 3 61 kcal g^{-1}, ME (Fig 1)

The body compositon, and nutrient retention efficiencies were markedly affected by the level of carbohydrate intake (Table 3) With increase in dietary carbohydrate from 8 to 48%, organic matter, body lipid and energy content in fish increased significantly (P < 0 05) over their initial values The moisture content was found to decrease significantly ($P < 0\ 05$) with increase in dietary carbohydrate The inorganic matter (ash) and crude protein content were not affected ($P > 0\ 05$) with the levels of carbohydrate in the diet The relationship of dietary carbohydrate with body constituents and nutrient retention efficiencies have been given in Table 4

Protein, fat and energy retention efficiencies increased linearly up to 40% dietary carbohydrate intake but beyond this level a significant ($P < 0\ 05$) fall in the values was noticeable (Fig 2)

DISCUSSION

In general, warmwater fish have no true carbohydrate requirement, but incorporation of certain level of carbohydrate in the diet influences conversion efficiencies and overall growth of fish (NAS - NRC, 1983), as is also evident from the present study on *C. catla* fry An increase in dietary carbohydrate from 8 - 40%, corresponding to 2 56 to 3 57 kcal g^{-1} (as ME), improved growth and conversion efficiencies These findings conform with the observations of Sen *et al* (1978) on spawn, fry and fingerling of *C mrigala* Similar phenomenon has been reported in common carp, red seabream and yellowtail (Furuichi and Yone, 1980), and rainbow trout (Edwards *et al*, 1977) More recently, it was seen that fingerling *L. rohita* can grow faster on diets containing 30% dietary carbohydrate (Erfanullah and Jafri, 1993) In contrast, in cod, increasing the amount of carbohydrate from 0 - 30% had reportedly no influence on weight gain, and protein and fat retention values (Hemre *et al*, 1989) It has been suggested that the capability of a species to adapt to higher carbohydrate levels in the diet depends on its ability to convert excess energy to lipid or non-essential amino acids (Tacon, 1990) Kaushik *et al.* (1989[a]) have maintained that the ability of fish to utilize carbohydrate may have both digestive and metabolic origin

The observations on FCR and PER values indicate that energy inclusion (through carbohydrate) up to a certain level (3 57 kcal g^{-1}, ME) in the diet increases weight gain, feed efficiency, and protein utilization The performance of the diet at the above level of carbohydrate inclusion (40%) reflects a proper nutrient balance in diet, and greater use of protein for growth purposes The sparing action of carbohydrate on protein has also been pointed out in other fish species (Jauncey, 1982, NAS-NRC, 1983, Piper *et al.*, 1989, and Tacon, 1990) A corollary to the pattern of changes observed in FCR and PER of *C. catla* fry fed increasing levels of energy (through carbohydrate) is evident in chinook salmon (Buhler and Halver, 1961), rainbow trout (Bergot, 1979[a], and Pieper and Pfeffer, 1980), plaice (Cowey *et al.*, 1975), walking catfish fry (Mollah and Alam, 1990), and common carp (Ufodike and Matty, 1983) A fall in FCR and PER with higher energy intake through carbohydrate could be attributed to decreased feed consumption owing to high dietary energy density and consequential low protein intake Since fish eat to satisfy their daily energy needs, supplying excess dietary energy may fulfill their energy requirements, before necessary amounts of protein or other nutrient requirements are met It is interesting to note that at 48% dietary carbohydrate the contribution of protein calories (as ME) is only 42%, which seems insufficient to meet the optimum protein needs of the fish The depressed growth and overall poor feed conversion efficiencies at 48% carbohydrate level point to the fact that *C. catla* is unable to handle excess dietary carbohydrate component Similar retardation in growth, due to increased proportion of carbohydrate energy, was reported for rainbow trout (Edwards *et al.*, 1977), common carp, red seabream and yellowtail (Furuichi and Yone, 1980).

The reduction in growth rate noted at 48% carbohydrate inclusion in the diet may also be attributed to altered energy to protein ratio (E/P) in the diet. The ratio varied from 8.92 kcal g^{-1} protein (in 40% carbohydrate diet) to 9.52 kcal g^{-1} protein (in 48% carbohydrate diet). In a preliminary experiment on fingerling *L. rohita*, optimum fish growth was similarly noted at an E/P ratio of 8.83 kcal g^{-1} protein in the diet (Erfanullah and Jafri, 1993). The value also compares favourably with that reported for common carp (Takeuchi *et al.*, 1979b).

Carcass composition was significantly affected by dietary carbohydrate intake in *C. catla*. With increase in dietary carbohydrate content from 8 - 48%, percent dry matter, body lipid and energy content of fish increased markedly, whereas inorganic matter (ash) and body crude protein percentages remained unaffected. These changes in carcass composition compare favourably with the results reported for rainbow trout (Bergot, 1979a, and Pieper and Pfeffer, 1980), European eel (Degani and Viola, 1987), common carp, red seabream and yellowtail (Furuichi and Yone, 1980), mirror carp (Ufodike and Matty, 1983), and tilapia (Anderson *et al.*, 1984).

Highest protein, fat and energy retention values occurred in fish at 40% dietary carbohydrate level but further increase in carbohydrate resulted in a significant ($P < 0.05$) fall in these values.

The overall low growth and poor feed conversion efficiencies noted in the fish at low dietary carbohydrate level (8%) could be the result of insufficient non-protein energy source in the diet necessitating greater utilization of dietary protein for purposes other than growth Since this diet also contained a high level of indigestible fibre in the form of α-cellulose, less efficient absorption and reduced availability of other necessary dietary nutrients seem understandable

In conclusion, the results of the present study indicate that in *C. catla* fry, 40% carbohydrate in a 40% CP diet, corresponding to an E/P ratio of 8 92, with 3 57 kcal g^{-1}, ME, produces maximum growth, best conversion efficiencies and higher nutrient retention Diets with either low (8%) or high (48%) levels of carbohydrate may reduce growth and conversion efficiencies, and affect nutrient retention and carcass composition

SUMMARY

Effects of varying levels of dietary carbohydrate (bread flour) on the growth, conversion efficiency, body composition and nutrient retention efficiency of *C. catla* (3 0±0 5 cm, 0 28±0 02 g) fry was investigated Isonitrogenous (40% CP) experimental diets with varying levels of carbohydrate (8,16,24,32,40 or 48%), were fed to triplicate groups of thirty fish each in 70 l high density polyvinyl flow-through (1-1 5 l/min) type indoor circular troughs (water volume 55 l) In a 6-week growth trial, fish were fed to apparent satiation, six days a week, twice daily at 0800 and 1600 h Weight gain (%) was significantly *(P 0.05)* affected by carbohydrate intake, resulting in a quadratic growth pattern Second degree polynomial regression analysis indicated that maximum SGR (3 41%) would occur at 37 69% carbodydrate

level, with an E/P ratio of 9 03 kcal g^{-1} protein and 3 61 kcal g^{-1} ME diet The relationship of dietary carbohydrate (up to 40 % inclusion) with SGR, PER and nutrient retention was linear and positive However, this relationship was negative with FCR Dietary carbohydrate significantly *(P 0.05)* altered carcass moisture, crude fat and gross energy content while crude protein and ash remained unaffected

Table 1. Ingredient and proximate composition of experimental diets.

Ingredients (g/100g, as fed basis)	Dietary 8	16	Carbohydrate 24	32	Levels (%) 40	48
Basal premix[1]	45.10	45.10	45.10	45.10	45.10	45.10
Soybean meal (49% CP)	18.12	15.84	13.55	11.30	8.98	6.69
Bread flour (14% CP)	8.00	16.00	24.00	32.00	40.00	48.00
a-cellulose	28.78	23.06	17.35	11.60	5.92	0.21
			Nutrients (% dry matter)[2]			
Crude protein	40.04	40.06	40.00	40.00	40.00	40.01
Crude fat	5.60	5.62	5.61	5.61	5.61	5.61
Ash	7.43	7.40	7.41	7.42	7.41	7.43
Crude fibre	35.63	28.81	23.29	16.85	10.42	4.35
NFE[3]	11.30	18.81	23.69	30.13	36.56	42.60
			Energy (kcal. g^{-1})			
Gross energy[4]	3.53	3.74	3.95	4.15	4.36	4.56
Metabolizable energy[5]	2.56	2.83	3.05	3.31	3.57	3.81
P:C:L (as % ME)[6]	63:18:19	57:26:17	52:31:17	48:37:15	45:41:14	42:45:13
E/P (as ME)	6.39	7.07	7.63	8.28	8.92	9.52

[1] Basal premix (g/100g): Casein, 19.04 (84%CP); gelatin, 4.56 (87.6%CP), fish meal, 14.50 (69%CP; 11% ash; 10% fat); corn oil, 4.00; Vitamin premix, 1.00; Mineral premix, 1.00 and carboxymethyl cellulose, 1.00g, respectively.

[2] Mean of triplicate runs ± SEM (N = 3).

[3] Nitrogen-free extract.

[4] Based on determined values on ballistic bomb calorimeter. GE contributed by the α-cellulose and CMC was subtracted, so that GE levels from ingredients are more truly represented.

[5] Based on 4.00, 4.00 and 9.00 kcal. g^{-1} for protein, carbohydrate and oil, respectively (Garling and Wilson, 1977).

[6] Protein, carbohydrate and lipid, respectively.

Table 2. Growth, conversion efficiencies, and survival (%) in *C. catla* fed varying levels of dietary carbohydrate*.

Diets (%CHO)	Mean initial body weight (g)	Mean final body weight (g)	Weight gain (%)	Specific growth rate (%)	Feed intake (mg/fish day^{-1})	Feed conversion ratio	Protein efficiency ratio	Survival (%)
8	0.28±0.12	0.67±0.17^{a}	138.22±16.60^{a}	2.07±0.06^{a}	24.29±6.1^{a}	3.48±0.13^{e}	0.72±0.03^{a}	94
16	0.29±0.10	0.87±0.18^{b}	200.78±10.30^{b}	2.60±0.06^{b}	28.75±2.12^{b}	2.77±0.05^{d}	0.90±0.02^{b}	96
24	0.29±0.10	0.97±0.16^{c}	240.48±11.23^{c}	2.91±0.07^{c}	31.79±7.1^{c}	2.62±0.06^{c}	0.95±0.02^{b}	96
32	0.29±0.10	1.14±0.14^{d}	291.45±19.30^{d}	3.25±0.05^{d}	37.52±7.9^{d}	2.44±0.01^{b}	1.03±0.01^{c}	98
40	0.29±0.10	1.26±0.15^{e}	338.58±19.56^{e}	3.52±0.07^{e}	40.89±6.11^{e}	2.35±0.01^{a}	1.13±0.02^{d}	98
48	0.28±0.10	0.95±0.14^{c}	240.70±19.54^{c}	2.92±0.06^{c}	31.96±2.24^{c}	2.67±0.03^{c}	0.94±0.01^{b}	98

* Results are mean of triplicate runs ± SEM $N = 90$ fish each/dietary treatment. Values in the same column not sharing the same same superscripts are significantly different ($P < 0.05$).

Table 3. Body composition and nutrient retention effeciencies in *C. catla* fry varying levels of dietary carbohydrate*

Diets (% CHO)	Body composition (% wet weight)					Nutrient retention efficienceis (%)[1]		
	Moisture**	Crude protein	Lipid	Ash	Energy** (kcal g^{-1})	Protein	Fat	Energy
Initial[1]	83.14 ± 0.41^{g}	12.26 ± 0.11^{a}	1.50 ± 0.09^{a}	2.92 ± 0.04^{d}	5.03 ± 0.03^{a}	-	-	-
8	82.07 ± 0.54^{f}	13.86 ± 0.12^{b}	2.19 ± 0.10^{b}	1.66 ± 0.06^{a}	5.62 ± 0.04^{c}	10.79 ± 1.62^{a}	13.84 ± 1.32^{a}	49.36 ± 2.18^{a}
16	81.94 ± 0.32^{e}	13.89 ± 0.12^{b}	2.36 ± 0.10^{c}	1.58 ± 0.08^{b}	5.68 ± 0.05^{d}	13.32 ± 1.18^{b}	18.05 ± 1.62^{b}	58.10 ± 2.18^{b}
24	80.17 ± 0.49^{d}	13.93 ± 0.14^{bc}	2.83 ± 0.14^{d}	1.93 ± 0.09^{c}	5.58 ± 0.06^{b}	13.90 ± 1.82^{c}	23.03 ± 1.35^{c}	56.16 ± 1.52^{b}
32	79.22 ± 0.30^{c}	13.86 ± 0.11^{b}	3.58 ± 0.16^{e}	1.81 ± 0.07^{c}	5.72 ± 0.07^{c}	14.78 ± 1.50^{d}	31.44 ± 1.52^{d}	58.84 ± 1.48^{c}
40	78.19 ± 0.40^{b}	13.90 ± 0.11^{b}	4.26 ± 0.12^{f}	1.88 ± 0.05^{c}	5.79 ± 0.04^{e}	15.97 ± 1.62^{e}	40.14 ± 1.28^{f}	61.31 ± 2.03^{d}
48	77.74 ± 0.89^{a}	13.84 ± 0.11^{b}	4.49 ± 0.11^{g}	1.96 ± 0.03^{c}	5.79 ± 0.03^{e}	13.56 ± 1.81^{b}	38.07 ± 1.81^{e}	50.08 ± 1.65^{a}

* Results are mean of triplicate runs ± SEM N=12 fish each/dietary treatment. Values in the same column not sharing the same superscripts are significantly different ($P < 0.05$).

** Dry matter basis.

[1] N = 35 fish.

Table 4. The relationships of dietary carbohydrate intake with body constituents and nutrient retention efficiency in *C. catla* fry.

Dietary carbohydrate to	r	n	$P < 0.05$
Body constituent			
Moisture	-0.987	21	0.05
Crude protein	0.602	21	n.s.
Fat	0.990	21	0.05
Ash	-0.384	21	n.s.
Energy	0.777	21	n.s.
Nutrient retention efficiency			
Protein	0.966	15	0.05
Fat	0.987	15	0.05
Energy	0.860	15	n.s.

n.s., not significant.

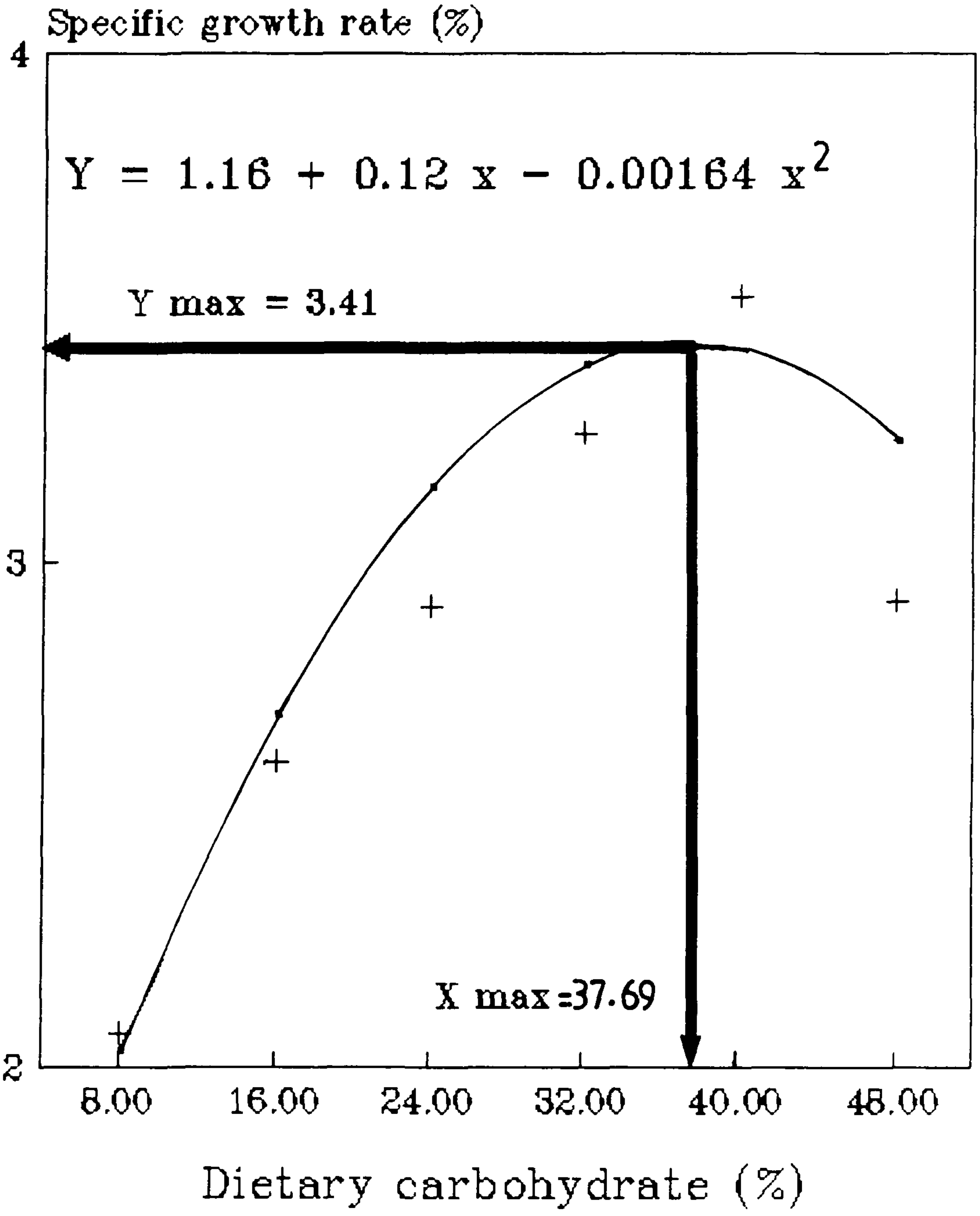


Fig. 1.
The second degree polynomial relation of SGR (%) and dietary carbohydrate in *C. catla* fry

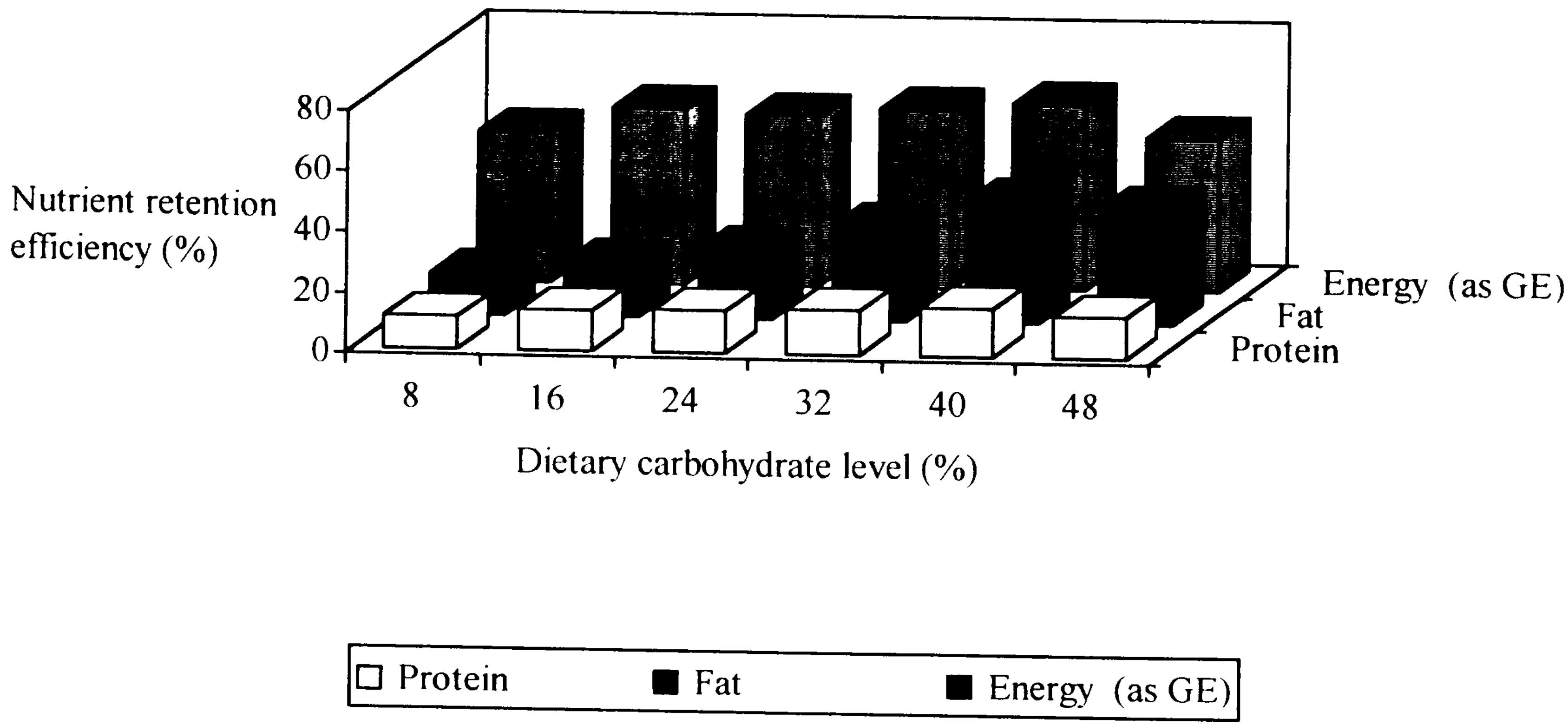


Fig. 2.
Nutrient retention efficiencies in *C. catla* fed varying levels of dietary carbohydrate

Chapter
V

CHAPTER V

PROTEIN-SPARING EFFECT OF DIETARY CARBOHYDRATE IN DIET FOR FINGERLING *LABEO ROHITA* (HAM.)

INTRODUCTION

Fish do not seem to have a true protein requirement, but a well-balanced mixture of indispensable amino acids in the diet is essential for their growth and maintenance In fish, gross protein requirement is known to vary with factors like species, the stage of growth, water temperature, salinity, protein to energy balance, digestibility of protein, and the amount of non-protein energy sources in the diet (NAS - NRC, 1983 , Wilson and Halver, 1986 , and Cowey, 1988) Several studies in the past have shown that provision of adequate levels of non-protein energy sources (carbohydrates and lipid) in the diet can minimize the use of protein as an energy source (Cho and Kaushik, 1990) Compared to lipid, the use of carbohydrate as a protein sparing energy source has received little attention (Lee and Putnam, 1973 , Cowey *et al.*, 1975 , Adron *et al.*, 1976 , Ogino *et al.*, 1976 , Edwards *et al.*, 1977 , Viola and Rapport, 1979 , Pieper and Pfeffer, 1979 , Takeuchi *et al.*, 1979[a] , Garcia-Gallego *et al*, 1981, 1993[a,b],Degani, 1987[a], Degani and Viola, 1986, 1987, Watanabe *et al*, 1987 , Serrano *et al.*, 1992 , Sanz *et al.*, 1993 , and Shiau and Peng, 1993) A knowledge of the optimal level of protein and protein-sparing effects of dietary carbohydrate may be useful in reducing the cost of fish feed considerably

The present study was undertaken to evaluate the effects of different levels of dietary carbohydrate (glucose, sucrose or dextrin), at variable protein levels, on the growth, conversion efficiency, nutrient retention, and body composition in fingerling *L. rohita*

MATERIALS AND METHODS

Experimental diets

Nine different iso-caloric (4 35 kcal g^{-1}, GE , 3 53 kcal g^{-1}, ME) diets were prepared to contain three levels (40, 35 and 30%) of crude protein At each protein level, three levels (30, 35 and 40%) of carbohydrate (glucose, sucrose or dextrin) were incorporated The ingredient and proximate composition of experimental diets is shown in Table 1 Gross energy content in the diets was determined on Gallenkamp ballistic bomb calorimeter, while metabolizable energy was calculated on the basis of physiological fuel values as described elsewhere (page 19)

Method of preparation of the experimental diet has been described on page 14 Proximate composition of the diets was determined according to standard methods (page 16 - 19)

Feeding trial

Source of fish, their acclimation, and details of general experimental design have been given elsewhere (page 12, 15)

L. rohita (4 1 ± 0 7 cm , 2 03 ± 0 01 g) were sorted out from the acclimated fish lot and stocked randomly, in triplicate groups of 30 fish each, in 70 l high density polyvinyl flow-through (1-1 5 l/min) circular troughs (water volume 55 l)

Fish were fed the experimental diets in the form of crumbles, six days a week, twice daily, at 0800 and 1600 h, to apparent satiation Feeding trial was conducted for eight weeks Initial and subsequent weekly weights (g) were recorded on a sensitive top pan balance, after anaesthetizing the fish with MS 222 solution (1 10,000) Average water temperature and dissolved oxygen, based on daily measurements, over the experimental period were 27 ± 1 C and 6 6 ± 0 2 ppm, respectively

Growth parameters and feed utilization efficiencies were measured using standard definitions (page 21 - 22)

Proximate composition and gross energy analysis

Initial proximate analysis and gross energy estimate in carcass were made, in triplicate, on fish randomly taken out from the acclimated stock using standard techniques (page 16 - 19) On termination of the feeding trial, required number of fish were taken out from each trough and analyzed similarly for final carcass and gross energy composition

Statistical analysis

Comparisons among treatment means were made by one-way analysis of variance (*ANOVA*) and Duncan's multiple range test ($P < 0\ 05$)

RESULTS

The growth and conversion efficiencies of *L. rohita* fed diets containing different levels of protein and carbohydrate are shown in Table 2 Fish receiving the dextrin containing diets gained significantly ($P < 0.05$) more weight (%) than those fed sucrose or glucose containing diets. Similarly, fish receiving the sucrose based diets grew faster than those fed the glucose containing diets ($P < 0.05$). Food conversion (FCR) was higher in fish fed dextrin containing diets in comparison to groups fed sucrose or glucose diets. An increase in dietary glucose inclusion from 30 - 40%, and a decrease in dietary crude protein from 40 - 30%, produced poorer food conversion ratios. Protein efficiency ratio (PER) improved with increase in dietary sucrose or dextrin contents from 30 - 40%, with a concomitant decrease in crude protein content of the diet from 40 - 30%, the highest PER being at 40% dextrin and 30% crude protein levels. However, the lowest PER occurred in fish fed the glucose based diets. Highest values for specific growth rate (SGR%) were seen with dextrin containing diets, followed by sucrose based diets. In fish fed glucose containing diets, SGR declined with incremental glucose levels.

Marked difference was noticeable in the body composition of fish fed different test diets (Table 3). At each protein level, with greater complexity of dietary carbohydrate, body dry matter, crude protein, total lipid and gross energy increased significantly ($P<0.05$). Ash content remained low showing no discernable change among fish fed the different test diets. Increasing the levels of sucrose and dextrin in the diet produced significantly higher ($P<0.05$) protein and energy retention values, the maximum being at 40% dextrin and 30% crude protein. However, increase in the dietary glucose depressed both protein and energy retention efficiencies significantly ($P < 0.05$).

DISCUSSION

It has been established that maximum growth and efficient protein utilization could be achieved in fish if,in addition to protein, other sources of energy are properly incorporated and utilized Results of the present study indicate that in *L. rohita* fingerling, weight gains, FCR, and SGR remained unaffected ($P > 0$ 05) when dextrin was used as a source of dietary carbohydrate, and its level increased from 30 - 40%, with a concomitant decrease in dietary protein from 40 - 30% Conversely, when sucrose or glucose were fed as carbohydrate sources both weight gain and SGR got reduced ($P < 0$ 05) Thus, 40% dextrin inclusion at 30% crude protein level in the diet appeared to maximize the utilization of protein for growth Such sparing action of dietary carbohydrate on protein utilization has also been noted in European eel (Degani, 1987[a] , Degani and Viola, 1987 , and Hidalgo *et al*, 1993), channel catfish (Garling and Wilson, 1976), and rainbow trout (Pieper and Pfeffer, 1980 , and Garcia-Gallego *et al.*, 1993[a]) More recently, Shiau and Peng (1993) have indicated that, decreasing the dietary protein level from 28% to 24% by increasing starch or dextrin content in the diet from 37% to 41% did not reduce weight gain and feed efficiency ratio in tilapia, suggesting that starch or dextrin could spare some protein when dietary protein was low

The source and complexity of carbohydrate and the presence of carbohydrate metabolizing enzymes are known to influence carbohydrate utilization in fishes (NAS - NRC, 1983) Better performance of dextrin containing diets in *L rohita* seems logical since utilization of complex carbohydrates, in contrast to disaccharides and simple sugars, is reportedly much higher in herbivorous and omnivorous fishes (Furuichi and Yone, 1980 , 1982 , Shimeno *et al.*, 1981, Anderson *et al.*, 1984 ; Wilson and Poe, 1987 , Tung and Shiau, 1991, 1993; and Shiau and Peng, 1993). The poor performance noted for the glucose containing diet in fingerling *L. rohita* seems in agreement with the findings on other fish species (Furuichi and Yone, 1982 ; Hilton and Atkinson, 1982 , Buddington and Hilton, 1987 ; Wilson and Poe, 1987, Tung and Shiau, 1991, 1993; and Shiau and Peng, 1993), where, in contrast to complex carbohydrates, inclusion of glucose, considered the most digestible (>90%) carbohydrate, could not enhance growth or feed utilization. It has been emphasized that in all such fishes a large portion of the absorbed glucose probably gets excreted out before adequate insulin is available to facilitiate its tissue utilization as an effective energy source (Pieper and Pfeffer, 1980 , Furuichi and Yone, 1981 ; Hilton and Atkinson, 1982 ; and Buddington and Hilton, 1987) Alvarado and Robinson (1979), and Hokazono *et al.* (1979) have attributed poor growth performance of fishes fed glucose containing diet to inhibition of amino acid absorption by the intestine.

In the present study, it was seen that FCR values remained unaffected when dietary crude protein was reduced from 40 - 30% and dextrin employed as the carbohydrate source. Similar trend appeared with sucrose containing diet However, when glucose was used as dietary carbohydrate source, poor FCR was observed. This indicates that, irrespective of changes in dietary protein levels, dextrin and, up to some extent, sucrose can effectively be utilized by fish for energy purposes. Similar beneficial effect of carbohydrate inclusion on fish growth and food conversion efficiency was noted in European eel by Degani and Viola (1987), and Hidalgo *et al.* (1993) Rychly (1980), on the other hand, observed that decrease in dietary protein from 74 to 32%, with a concomitant increase in the levels of carbohydrate from 9 to

53 % adversely affected the feed gain ratios. In the present study on *L. rohita*, at each protein level, PER generally increased with levels of carbohydrate when dextrin or sucrose was used as a carbohydrate source. This also demonstrates the protein sparing action of dietary carbohydrate. However, this was not true with glucose incorporated diets where PER remained low (0.86 - 0.82), reflecting towards poor utilization of glucose for energy purposes. High PER in fish fed diets containing low protein and high carbohydrate have been reported for European eel (Degani, 1987[a] , Degani and Viola, 1987 , Sanz *et al.*, 1993, and Hidalgo *et al.*, 1993), and tilapia (Shiau and Peng, 1993). However, in rainbow trout, PER values reportedly decreased with decreasing protein and increasing carbohydrate levels in the diet (Rychly, 1980). Results of the present study also clearly indicate that maximal growth,as reflected by improved PER, in *L. rohita* fingerling could be obtained even at low dietary protein, provided the diet contains a sufficient level of desired carbohydrate. In *L. rohita* high SGR occurred with increasing levels of dietary carbohydrate (up to 40%), when dextrin was used as a carbohydrate source, but increasing glucose or sucrose to the same level in the diet lowered the SGR, at decreasing levels of dietary protein. Since no significant difference ($P > 0.05$) in SGR were seen in fish receiving different levels of dextrin containing diets, weight gain (g day^{-1}) and SGR were compared between the three weekly sub-group periods (Table 4 and Fig. 1 & 2). Interestingly, during the first three weeks, maximum weight gain and SGR were noticeable at 40% crude protein 30% dextrin against 35% crude protein 35% dextrin, and 30% crude protein 40% dextrin based diets. In subsequent weeks, compared to the other diets, weight gain and SGR declined at 40% crude protein 30% dextrin diet, indicating that requirement for protein tend to decrease with fish growth,

as also pointed out by Halver (1969), and Millikin (1982)

The wholebody composition of *L. rohita* was found to alter with the test diets fed (Table 3) Moisture increased with increase in dietary protein and showed an inverse relationship with carcass lipid as has also been reported for other fishes (Degani and Viola, 1987 , Hidalgo *et al.*, 1993, and Khan *et al* , 1993[b]) Body protein also increased with dietary protein and correlated negatively with moisture Similar observations were made in other fish species (Page and Andrews, 1973 , Degani and Viola, 1987, and Hidalgo *et al.*, 1993) Increase in lipid concentration at higher levels of dietary carbohydrate finds corollary in earlier work (Anderson *et al.*, 1984 , Degani and Viola, 1987, and Hidalgo *et al.*, 1993) Body ash content remained low, showing no discernable change with dietary treatment High protein and energy retention values were observed in *L. rohita* with high carbohydrate and low protein diets, when dextrin or sucrose was used as a carbohydrate source In fish fed glucose containing diets, the protein and energy retention efficiencies decreased considerably with incremental carbohydrate level, indicating again that, compared to dextrin or sucrose, the ability of this species to utilize glucose as a non-protein energy source is low Improvement in protein and/or energy retention with increasing dietary carbohydrate, with a concomitant decrease in dietary crude protein, have also been reported in other fishes (Degani, 1987[a] , Degani and Viola, 1987 , Hidalgo *et al.*, 1993 , and Shiau and Peng, 1993) In rainbow trout, however, nitrogen retention values were reported to decline at low protein (32%) and high carbohydrate (53%), as compared to high protein (74%) and low carbohydrate (9%), inclusion in the diet (Rychly, 1980)

On the basis of PER and protein retention values, it can conclusively be stated that in fingerling *L. rohita*, a greater percentage of carbohydrate (dextrin or sucrose) gets utilized for energy purpose when fed diets with high carbohydrate and low protein content, sparing protein for growth This is further substantiated by the unaltered growth rate and feed efficiency values obtained with the above diet It has emerged that dextrin, as a carbohydrate source, is better utilized by this fish, and has a greater protein sparing-action than sucrose or glucose in the diet Results of the present study are important in formulating low-protein practical diets for the culture of this species

SUMMARY

Protein-sparing effect of different dietary carbohydrates was examined in fingerling *L rohita* (4 10±0 7 cm, 2 03±0 01 g) In a 8-week growth trial, nine different iso-caloric (4 35 kcal g^{-1}, GE , 3 53 kcal g^{-1}, ME) test diets, containing three levels of crude protein (40,35,30%) with three levels (30,35,40%) of different carbohydrate sources (glucose, sucrose or dextrin), were fed to triplicate groups of thirty fish each in 70 l high density polyvinyl flow-through (1-1 5 l/min) indoor circular troughs (water volume 55 l) Fish were fed to satiation, six days a week, twice daily at 0800 and 1600 h The result indicated that wieght gains (%), FCR, PER and SGR (%) remained unaffected *(P 0 05)* on increasing the carbohydrate inclusion from 30-40%, with a concomitant decreasing dietary protein level from 40-30%, when dextrin was used These values were moderate with sucrose based diets

However, poor values for the above were noticeable in fish fed glucose containing diets Marked difference was noticeable in the body composition of fish fed different test diets At each protein level, with greater complexity of carbodydrate, body dry matter, crude protein, total lipid and gross energy increased significantly *(P 0.05)*, while body ash content remained low showing no discernable change with dietary treatments Increase in the levels of sucrose and dextrin in the diet produced significantly higher *(P 0.05)* protein and energy retention values, while these values significantly *(P 0.05)* decreased with incremental glucose levels in the diet The study suggests that dextrin, as a carbodydrate source, was better utilised by *L.rohita* and had a greater protein-sparing action in the diet than sucrose or glucose

Table 1. Ingredient and proximate composition of experimental diets.

(g/100 g, as fed basis)	40% CP : 30% CHO			35% CP : 35% CHO			30% CP : 40% CHO		
	Glucose	Sucrose	Dextrin	Glucose	Sucrose	Dextrin	Glucose	Sucrose	Dextrin
Casein (84% CP)	28.57	28.57	28.57	23.81	23.81	23.81	19.05	19.05	19.05
Gelatin (87.6% CP)	6.85	6.85	6.85	5.71	5.71	5.71	4.57	4.57	4.57
Glucose	30.00	-	-	35.00	-	-	40.00	-	-
Sucrose	-	30.00	-	-	35.00	-	-	40.00	-
Dextrin	-	-	30.00	-	-	35.00	-	-	40.00
a-cellulose	5.32	5.32	5.32	6.22	6.22	6.22	7.12	7.12	7.12
Common ingredients[1]	29.26	29.26	29.26	29.26	29.26	29.26	29.26	29.26	29.26
Proximate composition (%)[2]									
Moisture	5.92	6.05	5.97	5.47	5.35	5.37	5.04	5.03	5.04
Crude protein	41.09	41.03	41.10	36.14	36.00	36.10	31.60	30.67	31.44
Lipid	7.09	7.11	7.05	7.10	7.11	7.10	7.07	7.05	7.07
Ash	5.45	5.42	5.38	5.21	5.32	5.29	5.11	5.14	5.04
Fibre	9.41	9.39	9.42	10.32	10.11	10.07	11.04	11.07	11.10
NFE[3]	31.04	31.00	31.08	35.76	36.11	36.07	40.14	41.04	40.31
Energy (kcal. g^{-1})									
Gross energy[4]	4.39	4.40	4.39	4.36	4.36	4.34	4.32	4.31	4.28
Metabolizable energy[5]	3.52	3.52	3.52	3.52	3.52	3.53	3.51	3.50	3.51

[1]Common ingredients (g/100 g) : Fish meal, 14.71 (68% CP; 10% ash; 10% fat) Corn oil, 3.70; Cod liver oil, 1.85; Mineral premix, 4.00 (Halver, 1976); Vitamin premix, 1.00 (Halver, 1976) and Carboxymethyl cellulose, 4.00 g, respectively.
[2]Mean of triplicate runs
[3]Nitrogen -free extract.
[4]Based on estimated fuel values on adiabatic oxygen bomb calorimeter.
[5]Based on 4.00, 4.00 and 9.00 kcal.g^{-1} for protein, carbohydrate and oil, respectively (Garling and Wilson, 1977).

Table 2. Growth efficiencies and survival of *L. rohita* fed varying levels of protein and carbohydrates[1].

Diet (%CP : %CHO)	Initial body weight (g)	Final body weight (g)	Weight gain (%)	FCR	Feed intake (mg/fish day^{-1})	PER	SGR (%)	Survival (%)
40:30 Glucose	2.02±0.09	3.96±0.26^{c}	96.04±14.20^{bc}	2.87±0.40^{d}	99.29 ± 6.0^{a}	0.85±0.11^{a}	1.20±0.20^{c}	96^{a}
40:30 Sucrose	2.04±0.10	4.89±0.20^{f}	139.34±10.64^{de}	2.42±0.11^{bc}	123.04 ±7.7^{b}	0.99±0.08^{b}	1.56±0.11^{de}	98^{a}
40:30 Dextrin	2.04±0.11	6.74±0.23^{g}	230.96±8.50^{f}	1.68±0.23^{a}	141.79±10.2^{c}	1.45±0.31^{d}	2.14±0.31^{f}	96^{a}
35:35 Glucose	2.01±0.04	3.72±0.28^{b}	84.92±12.39^{b}	3.23±0.46^{e}	98.57±7.7^{a}	0.86±0.12^{a}	1.10±0.15^{b}	96^{a}
35:35 Sucrose	2.06±0.04	4.67±0.36^{de}	127.20±13.65^{d}	2.25±0.24^{b}	106.00±6.6^{a}	1.23±0.23^{c}	1.47±0.15^{d}	98^{a}
35:35 Dextrin	2.05±0.05	6.86±0.21^{g}	235.37±14.03^{f}	1.64±0.34^{a}	141.70±8.2^{c}	1.69±0.06^{e}	2.16±0.11^{f}	96^{a}
40:30 Glucose	2.01±0.04	3.31±0.28^{a}	64.78±11.16^{a}	3.93±0.54^{f}	91.96±5.2^{a}	0.82±0.11^{a}	0.89±0.15^{a}	98^{a}
40:30 Sucrose	2.04±0.09	4.49±0.32^{d}	119.61±17.20^{d}	2.28±0.46^{b}	100.00±7.8^{a}	1.41±0.27^{d}	1.40±0.19^{d}	98^{a}
40:30 Dextrin	2.04±0.12	6.86±0.21^{g}	236.28±3.00^{f}	1.63±0.27^{a}	141.07±7.9^{c}	1.98±0.11^{f}	2.16±0.15^{f}	98^{a}

[1]Results are mean of triplicate runs ± SEM. N=30 fish each/dietary treatment. Figures in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.

Table 3. Body composition and nutrient retention efficiencies in *L. rohita* fed varying levels of protein and carbohydrates[1].

Diet (%CP : %CHO)	Body composition (% wet weight basis)					Nutrient retention efficiency	
	Moisture*	Crude protein	Lipid	Ash	Energy (kcal. g^{-1} dry weight)	Protein	Energy
Initial fish	79.83±0.19	14.54±0.11	3.08±0.21	1.72±0.03	5.70±0.03	-	-
40:30 Glucose	77.17±0.11^{f}	14.43 ±0.10^{c}	3.92±0.11^{b}	1.80±0.32^{a}	5.74±0.19^{ab}	12.49±1.30^{a}	45.73±4.51^{b}
40:30 Sucrose	75.35±0.24^{d}	16.41±0.11^{f}	4.23±0.24^{c}	1.84±0.29^{a}	5.79±0.27^{ab}	17.89±1.20^{b}	55.03±2.20^{c}
40:30 Dextrin	74.11±0.13^{c}	17.82±0.13^{g}	5.18±0.29^{e}	1.81±0.24^{a}	5.98±0.32^{c}	27.83±0.94^{de}	82.51±1.00^{d}
35:35 Glucose	78.15±0.11^{f}	13.93±0.03^{b}	3.97±0.19^{b}	1.77±0.32^{a}	5.78±0.21^{ab}	11.34±1.14^{a}	41.75±4.00^{b}
35:35 Sucrose	75.56±0.21^{de}	16.12±0.16^{e}	4.39±0.19^{cd}	1.94±0.45^{ab}	5.79±0.16^{ab}	21.36±1.01^{c}	59.95±4.01^{c}
35.35 Dextrin	73.22±0.11^{b}	17.70±0.06^{g}	5.36±0.32^{e}	1.78±0.29^{a}	5.96±0.27^{c}	32.20±1.00^{f}	85.36±2.02^{d}
30:40 Glucose	80.10±0.16^{g}	13.14±0.19^{a}	3.47±0.24^{a}	2.19±0.29^{b}	5.63±0.27^{a}	9.03±1.62^{a}	32.96±3.20^{a}
30:40 Sucrose	74.18±0.13^{c}	15.89±0.21^{d}	4.38±0.24^{cd}	1.97±0.27^{ab}	5.71±0.21^{ab}	24.05±2.80^{cd}	58.57±8.01^{c}
30:40 Dextrin	72.10±0.11^{a}	17.68±0.48^{g}	5.79±0.48^{f}	1.80±0.32^{a}	5.98±0.29^{c}	37.45±1.50^{g}	87.01±3.21^{d}

[1]Means of 3 replicate groups ± SEM. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.

*Dry matter basis.

Table 4. Weight gain (g) and specific growth rate (%) of *L. rohita* fed variable protein and dextrin containing diets during the experimental period*

Diet % CP: % CHO	Weight gain (g)			Specific growth rate (%)		
	week			week		
	0-3	3-6	6-8	0-3	3-6	6-8
40:30 Dextrin	1.84 ± 0.04^{b}	1.82 ± 0.11^{a}	1.04 ± 0.09^{a}	3.07 ± 0.03^{b}	1.74 ± 0.15^{a}	1.20 ± 0.10^{a}
35:35 Dextrin	1.69 ± 0.11^{a}	2.01 ± 0.07^{b}	1.10 ± 0.07^{a}	2.89 ± 0.12^{a}	2.04 ± 0.09^{b}	1.25 ± 0.08^{a}
30:40 Dextrin	1.67 ± 0.04^{a}	2.04 ± 0.09^{b}	1.11 ± 0.14^{a}	2.84 ± 0.06^{a}	2.09 ± 0.06^{b}	1.27 ± 0.16^{a}

* Results are mean of triplicate runs ± SEM. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.

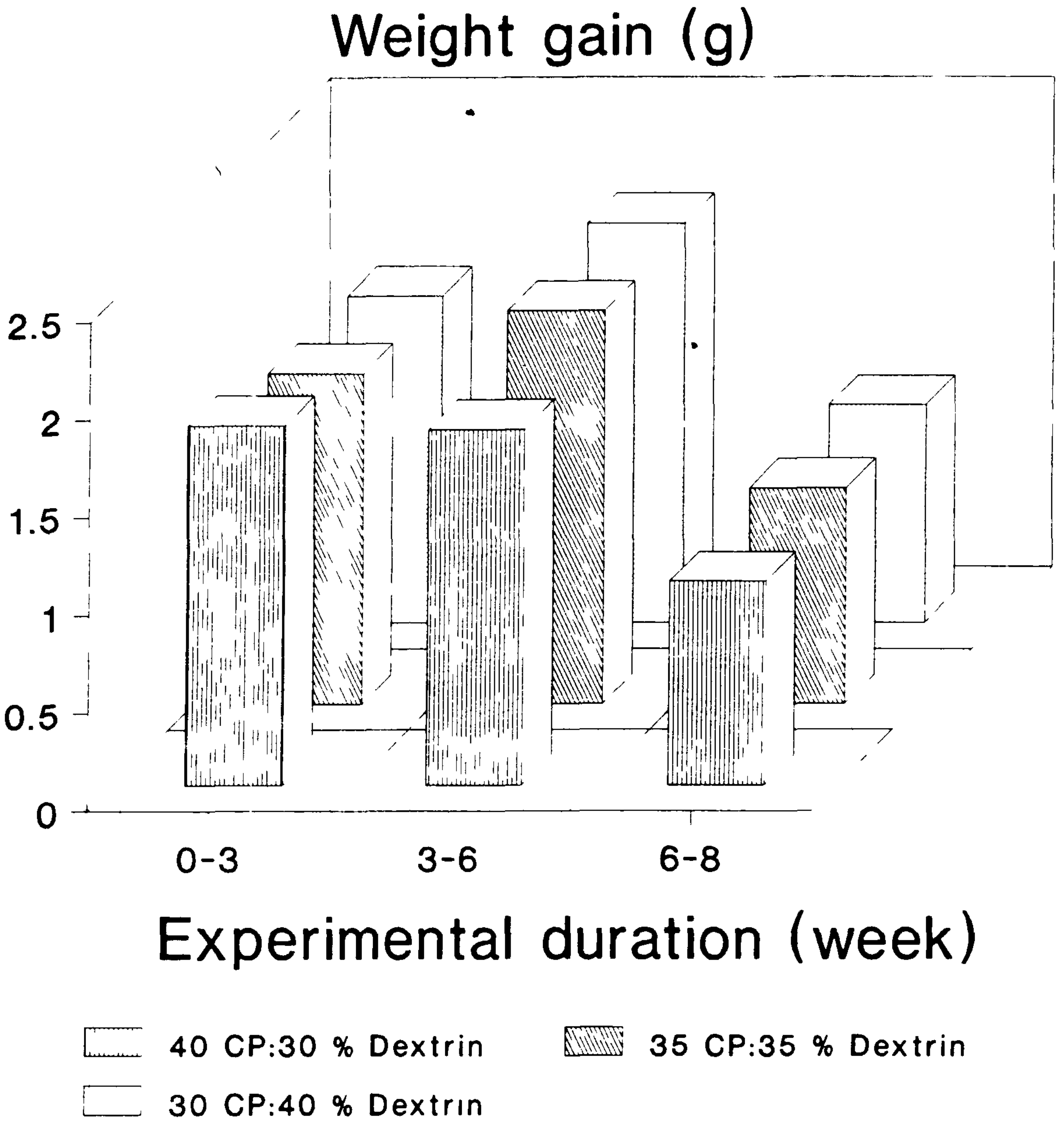


Fig. 1. Weight gain of *L. rohita* fed variable protein and dextrin containing diets during the experimental period.

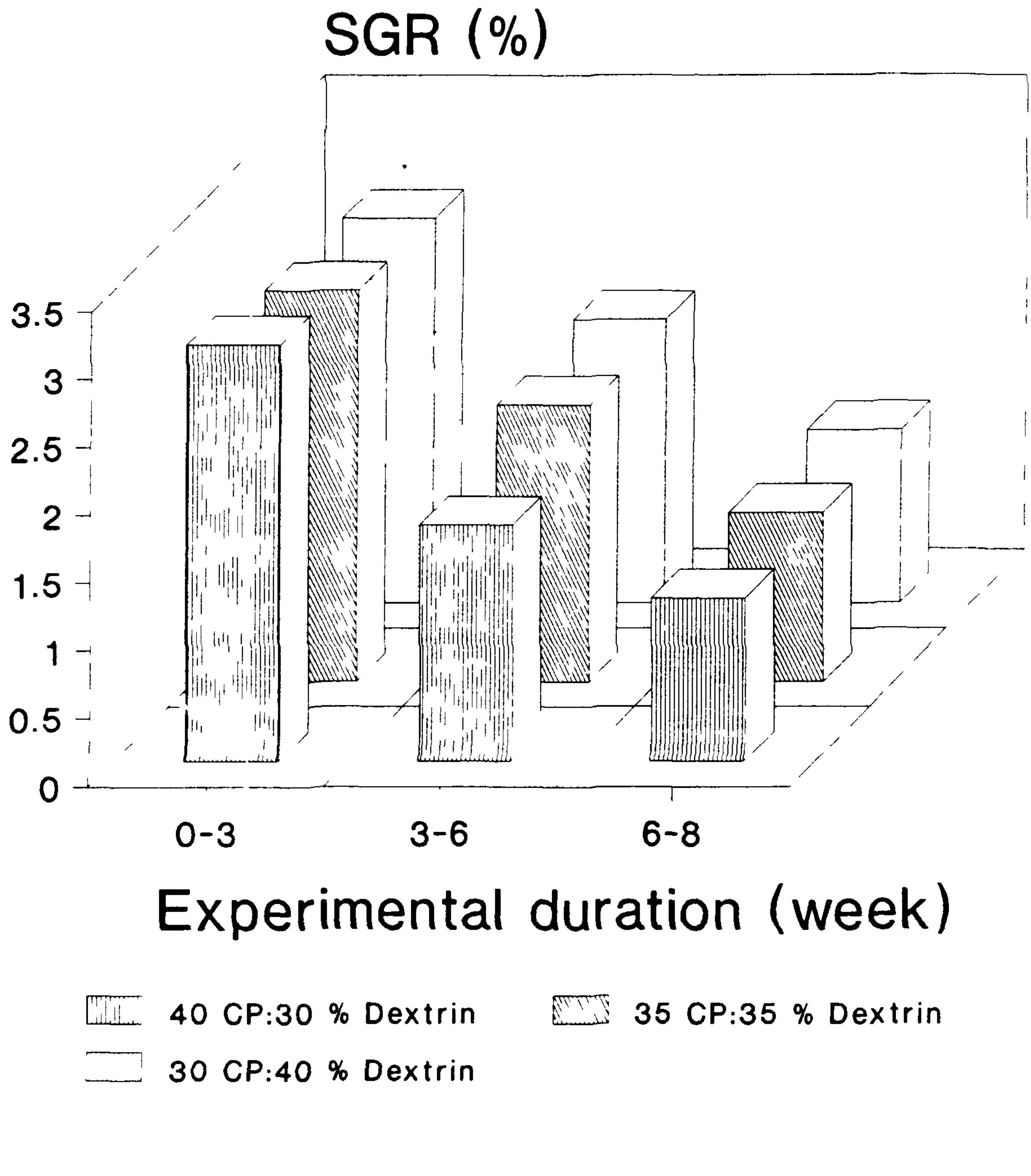


Fig. 2. SGR (%) of *L. rohita fed* variable protein and dextrin containing diets during the experimental period.

Part II

Chapter VI

CHAPTER VI

DRY MATTER, NUTRIENT AND ENERGY DIGESTIBILITY COEFFICIENTS IN SOME CARBOHYDRATE RICH FEEDSTUFFS FOR FINGERLING INDIAN MAJOR CARPS

INTRODUCTION

With the intensification of fish culture operation and constant increase in the cost of many of the conventional feedstuffs, the need to develop nutritive and economical feed,using locally available less expensive, agro-based by -products, is being increasingly felt (FAO/UNDP, 1979 , and Halver, 1982) In India, a wide variety of cost-effective, agro-based feedstuffs are available, and nutritional scanning indicates that a good number of these are rich in protein, carbohydrate and energy (Jafri *et al.*, 1992) However, very few of these feedstuffs have been evaluated for their apparent nutrient and energy digestibility (Nandeesha *et al.*, 1991, and Singh, 1991, 1992,) Since ability to digest nutrients and energy from a particular feedstuff varies with fish species, it is imperative to workout the nutrient and energy digestibility of different feedstuffs for optimum inclusion in formulated diets, to allow effective substitution of one ingredient by the other for achieving maximum growth

In the past, several studies were made to evaluate the dry matter, nutrient(s) and/or energy digestibility coefficients in a wide variety of feedstuffs/feeds fed to various fish species (Lovell, 1977, Kirchgessner *et al*, 1986 , Law, 1986 , Hanley, 1987, Martinez-Palacios, 1988 , Eid and Matty, 1989 , Hossain and Jauncey, 1989 , Hossain *et al.*, 1992, Kamarudin *et al*, 1989 , Singh, 1991,1992 , Cho, 1993, Hajen *et al.*, 1993[b],Dimes and Haard, 1994, Dimes *et al.*,1994[a,b], Khan, 1994, and Kaushik *et al.* 1994) Factors like size, age and density (Hastings, 1969 , and Windell *et al.*, 1978), feeding level and meal size (Windell *et al.*, 1978, and Henken *et al.*, 1985) , physical state of diet (Luquet and Bergot, 1976 , Bergot and Berque, 1983, Kaushik *et al*, 1989[a] , Jeong *et al.*, 1992[a,b] , Takeuchi *et al.*, 1992 , Arnesen and Krogdahl, 1993, Bergot, 1993 , and Bergero *et al.*, 1993), dietary components, protein, lipid, fibre, etc (Inaba *et al.*, 1963, Cho *et al.*, 1976, Rychly and Spannhof, 1979, Beamish and Thomas , 1984 , De Silva and Perera, 1984 , Spyridakis *et al.*, 1989 , De Silva *et al.*, 1988, 1990, Vergara and Jauncey, 1993, and Shiau and Liang, 1994), and degree of inclusion of the test ingredent in the diet (Spyridakis *et al.*, 1988 , De Silva, 1989, De Silva *et al.*, 1988, 1990 , Nandeesha *et al.*, 1991, Hajen *et al.*, 1993[b] , and Ray and Das, 1994) have also been identified to influence nutrient(s)/energy digestibility in fish

Indian major carps are generally fed on diets formulated on empirical basis, based on combinations of a few conventional feedstuffs such as rice bran and various varieties of oil cakes, mainly for supplementary feeding There is, however, increasing emphasis on development of low-cost, balanced artificial rations for these fishes, using less expensive, agro-based by-products Assessment of digestibility of such feedstuffs is considered essential for their effective incorporation in fish diets (Chow, 1982) This study evaluates the dry matter, nutrient and energy digestibility coefficients in some locally available, low-cost, carbohydrate rich feedstuffs, for fingerling Indian major carps, *C catla, L. rohita*, and *C. mrigala*, using chromic oxide as indigestible external marker The nutritional evaluation of carbohydrate rich feedstuffs is considered important while formulating optimum protein to energy ratio diet for the fish, particularly for young fish, where protein should be spared for growth purposes

MATERIALS AND METHODS

Experimental fish

Source of fish, their acclimation, and the details of general experimental design have been described elsewhere (page 12, 15)

Fingerling *C. catla* (4 0 - 6 0 cm, 1 5 - 2 5 g), *L. rohita* (3 9 - 6 2 cm, 1 5 - 2 6 g), and *C. mrigala* (4 1 - 6 0 cm, 1 6 - 2 8 g) were randomly stocked, in triplicate groups of 25 fish each (N = 75 fish/dietary treatment), in 70 l high density polyvinyl flow-through (1 - 1 5 l/min) circular troughs (water volume 55 l) in wet laboratory Fishes were acclimated to the reference diet for ten days

Feedstuffs

Nine different native and two steam-cooked carbohydrate rich feedstuffs, generally employed for supplementary feeding of carps, were used in the present study Samples of feedstuffs, purchased from the local market, were carefully ground to a fine powder and preserved in polythene bags for chemical analysis Yellow corn and potato were steam cooked at 15 lb/in^2 pressure for 30 min, dried in a hot-air oven (60° C) for 24 hours, and preserved and stored as above The proximate composition and gross energy (kcal g^{-1}) content of feedstuffs are given in Table 1

Experimental diets

The dry matter, nutrient and energy digestibility was estimated using the reference (40% crude protein, 1% chromic oxide) and test diets (Cho *et al*, 1985) Casein and gelatin were used as protein sources (Table 2) in the reference diet (RD) Use of the reference diet was made to ensure that the fishes obtain all essential nutrients, including essential amino acids, for normal growth according to their requirement for dietary protein (Khan and Jafri, 1991[a]) The composition of mineral and vitamin premixes were according to Halver (1976) Test diets consisted 30% test ingredient and 70% casein-gelatin based reference diet

The proximate composition of the reference and the test diets is shown in Table 3 Preparation of the experimental diet has been described under General Methodology Section (page 14)

Experimental trial

Prior to feeding the test diets, the fishes were acclimated to the reference diet for 10 days They were fed the reference diet to satiation daily at 0800 and 1600 h Each dietary treatment had 3 replicates, with 25 fish in each replicate The average water temperature and dissolved oxygen, based on daily measurements, over the experimental period were 27 ± 1 °C and 6 4 ± 0 3 ppm, respectively Fishes were acclimated to the test diets for 5 days before the start of faecal collection The reference and test diets were in the form of dry crumbles Any uneaten food was siphoned off 2 hours after each feeding Faecal collection was made daily before feeding between 0700-0800 and 1500-1600 h The collection was made over a fine polythene mesh through slow siphoning, using a narrow (8 00 mm) plastic tube Care was taken to avoid breakage of thin faecal strings, and to minimize nutrient leaching Faecal matter from each treatment group was pooled together in a clean properly sealed container and immediately frozen (-20° C) Faecal samples collected over three weeks were pooled for subsequent chemical analysis

Analytical methods

All chemical analysis were made in triplicate Test ingredients, feed and faecal samples were homogenized to powdered form, weighed and analyzed for proximate composition and gross energy content according to the standard methods (page 16 - 19) Chromic oxide was estimated with acid digestion technique (page 20) of Furukawa and Tsukahara (1966)

Calculation of digestibility coefficient

The apparent digestibility coefficient (ADC) for dry matter, nutrient and energy in the reference and the test diets were calculated as described by Maynard and Loosli (1969)

$$ADC = 100 - 100 \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in feed}} \times \frac{\%\ Cr_2O_3\ \text{in feed}}{\%\ Cr_2O_3\ \text{in faeces}}$$

The dry matter, nutrient and energy ADC in the test ingredient were calculated from digestibility coefficients for the reference and the test diets, on the basis of 30% substitution of the test ingredient for the reference diet, using the following formula (Cho *et al.*, 1982)

$$100/30\ \{\text{ADC of test diet} - 70/100\ \text{ADC of reference diet}\}$$

Statistical analysis

Data on the digestibility coefficient for dry matter, nutrient and energy estimates in the three fish species fed different or the same ingredient was subjected to one way analysis of variance Significant difference between treatment means were compared using Duncan's multiple range test at 0 05% level of probability

RESULTS

Data on proximate composition and gross energy content of feedstuffs used for digestibility estimate is shown in Table 1 Gross nutrient analysis of various feedstuffs reveal the nutritive richness of the feedstuffs, in terms of energy, carbohydrate and other nutrients The moisture content in the feedstuffs ranged between 5-7 %, while crude protein and lipid levels varied between 5 - 12% and 0 13 - 12%, respectively Ash content varied between 1 4 - 13% Among the feedstuffs, Bengal gram dust (BD), soybean husk (SBH) and lentil husk (LH) contained higher crude fibre, 35, 31 and 20% , respectively, while in the remaining feedstuffs, the fibre content ranged between 2 - 11% Appreciable amount of carbohydrate, ranging between 43 - 83% ,were contained in the feedstuffs The gross energy content varied from 3 5 - 4 6 kcal g^{-1}

The proximate analysis, gross energy, and percent chromic oxide of faecal matter of fish fed the reference and the test diets are given in Table 4 ADC for dry matter, energy, and nutrient (protein, lipid, carbohydrate) for these diets are given in Table 5

The values of digestibility coefficient for the dry matter and nutrient components of the test ingredients are given in Table 6. The data indicates that, within each test species, namely, *C. catla*, *L. rohita*, and *C. mrigala*, the dry matter and nutrient digestibility coefficients varied significantly ($P < 0.05$) with feedstuffs tested. However, with few exceptions, digestibility coefficients for dry matter and nutrients for an individual test ingredient varied insignificantly ($P > 0.05$) among the three species (Figs. 1-5). In these fishes, higher values for dry matter digestibility (94 - 96%) were found with cooked potato starch diet (CPS). Raw potato starch diet (RPS) produced significantly ($P < 0.05$) lower dry matter digestibility (74%) in *C. catla*, and *L. rohita*. In *C. mrigala*, the lowest dry matter digestibility (75%) was obtained with barley dust diet (BD). No significant difference ($P > 0.05$) was noted in dry matter digestibility between SBH, LH and WB, RB and CYC, and RP and BD in *C. catla*, RP and RYC, and WB and SBH in *L. rohita*, and RB and CYC, WB, SBH and LH, and RP and BGD in *C. mrigala*.

The digestibility coefficient for crude protein was fairly high, ranging from 93% in WB to 97% in CPS. The values for other feedstuffs fell between 83 and 90% in *C. catla*. In *L. rohita*, higher values were recorded for WB (93%) and CPS (97%), while for other feedstuffs the values ranged between 81 and 90%. In *C. mrigala*, higher values were found for CYC (96%) and CPC (98%), values for other feedstuffs oscillated between 78 and 93%.

Within the three species of Indian major carps, compared to other feedstuffs, lipid digestibility values were significantly lower ($P < 0.05$) for BD, LH, SBH, BGD, and RPS, ranging between 69 - 81%.

Within each species, significant difference ($P < 0.05$) in carbohydrate digestibility could be observed between cooked (CYC and CPS) and raw carbohydrates. However, lowest ($P < 0.05$) carbohydrate digestibility for the three species were observed with RP. Similarly, values for energy digestibility were significantly higher ($P < 0.05$) for cooked (CYC and CPS) than for raw carbohydrates.

DISCUSSION

Gross nutrient analysis of feedstuffs, in terms of energy, carbohydrate and other nutrients, indicate that several of the low-cost feedstuffs may be used as effective source of energy and nutrients in practical diets for cultured fish. The estimated proximate composition and energy density of these feedstuffs compare favourably with the values reported earlier (Cullison, 1978 ; Ensminger and Olentine, 1980 ; NAS - NRC, 1981, 1983 ; and Jafri *et al.*, 1992).

Information on the nutrient digestibility of feedstuffs in warmwater fish, particularly in carps, is limited (Hastings, 1969 ; NAS -NRC, 1983 ; Law, 1984 ,1986 ; Steffens, 1985; Kirchgessner *et al.*, 1986 ; and Hossain and Jauncey, 1989). With the exception of the work of Singh (1991, 1992), and Nandeesha *et al.* (1991) on energy, crude protein and lipid digestibility from various feedstuffs and pelleted diets, in *C. mrigala*, *C. catla*, and grass carp, nutrient digestibility studies on Indian major carps are almost lacking.

The ADC for dry matter, energy and nutrients obtained for plant origin feedstuffs in *C. catla*, *L. rohita*, and *C. mrigala* during the present study appeared higher when compared to the values reported in fishes like tialpia (Hanley, 1987 ; Lorico-Querijero and Chiu, 1989 ; and Kamarudin *et al.*, 1989), and common carp (Kirchgessner *et al.*, 1986, and Law, 1984, 1986).

A comparison of the values obtained for dry matter digestibility, within the three major carp species, indicate that dry matter digestibility get affected with the source and complexity of carbohydrate, as also with the fibre content of the feedstuff and the test diet Diets containing higher levels (10 - 15%) of dietary fibre such as BGD, SBH and LH showed low values of dry matter digestibility compared to diets with lower levels (5 - 8%) of dietary fibre Further, in fish with higher values for dry matter digestibility, the amount of faecal collection was the lowest Low dry matter digestibility noted for raw carbohydrate source (RYC and RPS) in the major carps was similarly reported in rainbow trout (Kaushik *et al.*, 1989[a])

The digestibility coefficients for protein component indicate that all the three species of major carps are equally effective in digesting protein, irrespective of the feedstuffs (carbohydrate) used Since the test diets were almost isonitrogenous (30 - 32% CP), the variations observed in protein digestibility with certain feedstuffs could partly be attributed to the nature and composition of these feedstuffs Decreased protein digestibility noted for BGD, SBH, and RB could be the result of increased fibre and ash content Interestingly, these feedstuffs and the test diets, that contained higher fibre and ash content, showed significantly ($P < 0.05$) low protein digestion compared to feedstuffs and diets with lower levels of fibre and ash Evidence of negative effect of these constituents (fibre and ash) on protein digestibility is also apparent in the work of Kirchgessner and Schwarz (1982) on carp, Lorico-Querijero and Chiu (1989), and De Silva *et al.* (1990) on tilapia, Nandeesha *et al* (1991) on *C. catla*, and Vergara and Jauncey (1993) on gilthead sea bream Cooking raw carbohydrates, such as potato and corn, significantly ($P < 0.05$) improved the protein digestibility in the three species of the test fish. This may be ascribed to thermal modification in tertiary structure of protein (Peisker, 1992) or inactivation of protease inhibitors and other antinutritional factors in raw corn and potato (Jauncey and Ross, 1982; and Tacon and Jackson, 1985). In contrast, El-Sayed (1991) observed no significant improvement in the protein digestibility of tilapia fed cooked sugarcane bagasse. Similarly, in rainbow trout, protein digestibility coefficients were not found to vary significantly between raw and extruded carbohydrates (Kaushik *et al.*, 1989[a]).

As can be seen from the data, the values of ADC for lipid generally fell in three distinct categories with respect to feedstuffs analysed, relatively high (89 - 99%) for RB, RP, WB, CYC, CPC ; moderate (81 - 91%) for RYC, SBH, RPS ; and low (69 - 79%) for BD, LH and BGD. The variations are understandable since crude fat or ether extract repersents a very heterogeneous group of compounds, especially in plant origin feedstuffs. Similar variations in fat digestibility have earlier been reported in carp for plant origin feedstuffs (Kirchgessner *et al.*, 1986). It has also been observed by some workers that composition of fatty acids and melting point of fat have a strong bearing on fat digestibility (Takeuchi *et al.*, 1979[c] ; and Austreng *et al.*, 1980). Higher lipid digestibility values have been reported in carp , including *C. mrigala,* and tilapia fed oil seed based diets (Hossain and Jauncey, 1989 ; Singh, 1991 ; and Hossain *et al.*, 1992), and in carp fed grains and their by-products (Kirchgessner *et al.*, 1986). A significant improvement (10 -12%) in fat digestibility was observed in the processed (cooked) over native (raw) feedstuffs. Peisker (1992) has pointed out that during heat processing or extrusion, the operating temperature (140 - 150°C) inactivate microbial lipases which are reportedly produced by certain microbes (such as *Pencillium,* *Pseudomonas* and *Candida*, etc) and cause most of the decomposition of fat in feedstuff/compounded feed, resulting in poor fat digestion

The present study on the carbohydrate and energy digestibility clearly indicates that in major carps, carbohydrates from several agro-based by-products (brans/husks, dusts etc) and cooked starch are highly available to the test fish Starch from wheat, corn and potato are reported to be 85% digested by carp (Chiou and Ogino, 1975), while that from grains (wheat, barley, rye, oats and corn) and their by-products (corn and potato starch) produced 56 - 90% digestibility values of the total carbohydrate fraction in carp (Kirchgessner *et al.*, 1986) The digestion coefficients for carbohydrate and energy of maize meal was 71 and 56% in jelawat (Law, 1984), and 88 and 55% in carp (Law , 1986) In red tilapia, the carbohydrate and energy digestibility coefficient for corn meal and rice bran were reported to be 92 and 83%, and 82 - 88% , respectively (Kamarudin *et al.*, 1989)

Higher values for carbohydrate and energy digestibility noted during the present work point to the fact that Indian major carps thrive well on diets containing relatively higher levels of dietary carbohydrate (36 - 48%) Similarly, in carp, high levels of rice and cassava in diet (40%) promoted protein digestion (Ufodike and Matty, 1983) However, the variability of carbohydrate and energy digestion in the three species of major carps seemed more related to the source, complexity and degree of hydrolysis through starch gelatinization of carbohydrate since nitrogen-free extract contain both highly digestible carbohydrates as well as large amounts of indigestible lignin Moreover, the fibre fraction of the feedsuffs also contain other compounds such as cellulose, pentosane, hemicellulose, etc , which are reportedly indigestible in carp (NAS - NRC, 1977 , Bergot, 1981 , Kirchgessner and Schwarz, 1982 , Schwarz and Kirchgessner, 1982, Kirchgessner *et al.*, 1986, and Schwarz *et al.*, 1986), and rainbow trout and tilapia (Buddington, 1980) Digestible energy of cooked starch is also greatly enhanced due to gelatinization of starch The poor digestibility of native starch and the beneficial effects of cooked starch is well recognized in carp (Chiou and Ogino, 1975, and Shimeno *et al.*, 1977), channel catfish (Cruz, 1975, and Lovell, 1984), eel (Spannhof, 1976, and Spannhof and Kunhe, 1977), and rainbow trout (Cho and Slinger, 1979 , Bergot and Berque, 1983 , Kaushik and Oliva-Teles, 1985 , Kaushik *et al.*, 1989[a], and Hajen *et al.*, 1993[b])

The poor carbohydrate digestibility noted for RP and BGD in major carps may be the result of poor ingredient quality of the former and low palatability of the latter feedstuff As already pointed out, feeding fishes on diets such as RP, RYC, RPS, BGD and BD, with low carbohydrate and energy digestibility, resulted in maximal faecal output, indicating faster passage of chyme in the intestine, thus negatively affecting the carbohydrate and energy digestion Similar fact was observed earlier in rainbow trout fed raw starch (Spannhof and Plantikow, 1983)

The overall moderate to high dry matter, nutrient and energy digestibility noted in the three species of major carps fed various agro-based by-products and cooked starch may also be related to the omnivorous/ herbivorous feeding habit, and the enzyme equipment of these fishes The finding strengthens the view that the enzyme system in fishes with long gut is better equipped to digest and absorb nutrients from plant origin feedstuffs (Smith, 1989[b]) Relatively high values of amylase activity is reported in these carps (Dhage, 1968) Further, amylase, cellulase , protease and lipase activities were reported in the gut and hepato-pancreas of carps, and their activity found directly proportional to the amount of substrate in the food (Scherbina and Kazlaskene, 1971 ; Kawai and Ikeda, 1971 ; Scherbina *et al.*, 1976, Prejs and Blaszczyk, 1977; and Das and Tripathi, 1991). Species-wise comparison of the ability of the major carps to digest dry matter, nutrients and energy from the feedstuffs tested, clearly point to the possible similarity in their enzyme equipment. Thus practical rations could be formulated for the polyculture of these carps, using similar feedstuffs, provided their nutritional requirement matches each other. These findings are important in developing low-cost and balanced rations, with locally available agro-based feedstuffs, for the three species of the major carps.

SUMMARY

Evaluation of apparent dry matter, crude protein, fat, carbohydrate and energy digestibility coefficients in nine raw and two steam-cooked agro-based by-products in pelleted feed (70:30 reference : test ingredient), containing 30-32 % CP, 3.9-4.3 kcal.g^{-1} gross energy and 1 % chromic oxide, were carried out in fingerling Indian major carps, *C. catla* (4-6 cm; 1.5-2.5 g), *L. rohita* (3.9-6.2 cm, 1.5-2.6 g), and *C. mrigala* (4.1-6.0 cm; 1.6-2.8 g). Each dietary treatment had three replicates of twenty five fish each. Experimental trial was conducted in 70 l high density polyvinyl flow-through (1-1.5 l/min) indoor circular troughs (water volume 55 l). Fish were fed to apparent satiation, twice daily at 0800 and 1600 h. Faecal sanples were sipnoned daily at 0700 and 1500 h. The results indicated that, within each test species, apparent dry matter, nutrient (protein, fat and carbohydrate), and energy digestibility coefficients

varied significantly *(P 0.05)* with the feedstuff tested However, with few exceptions, digestibility coefficients for an individual test ingredient varied insignificantly *(P 0.05)* among the three fish species The study clearly indicates that feedstuffs rich in their carbohydrate and energy contents are effectively utilized by the three species of the Indian major carps The variations observed in dry matter, nutirient and energy digestibility coefficients in these species seemed related to the source and nutrient composition of the feedstuffs Feedstuffs and test diets with higher levels of fibre and ash showed significantly *(P 0.05)* low values for dry matter, nutrient and energy digestibility Steam-cooking of corn and potato significantly *(P 0.05)* improved the dry matter, nutrient and energy digestibility coefficients in the test species The findings of the present study are important in developing low-cost balanced rations, incorporating locally available agro-based by-products, for the polyculture of the three species of Indian major carps

Table 1. Proximate composition and energy content of test ingredients*.

Test ingredient	Moisture**	Crude protein	Crude fat	Ash	Fibre	Carbohydrate	Energy**
	(% dry matter)						kcal. g⁻¹
Rice bran (RB) (*Oryza sativa*)	5.07	12.05	1.83	13.37	10.85	56.83	3.50
Rice polish (RP) (*Oryza sativa*)	6.69	8.75	12.17	5.17	7.96	59.26	4.58
Wheat bran (WB) (*Triticum aestivum*)	4.54	10.45	3.97	6.94	9.13	64.97	4.52
Raw yellow corn (RYC) (*Zea maize*)	5.74	5.89	3.78	1.39	2.90	80.30	4.08
Cooked yellow corn (CYC) (*Zea maize*)	5.17	6.43	4.61	1.62	2.997	79.18	4.33
Raw potato starch (RPS) (*Solanum tuberosum*)	5.89	6.74	0.13	3.00	2.24	82.00	4.10
Cooked potato starch (CPS) (*Solanum tuberosum*)	5.34	6.52	0.14	3.16	1.934	82.91	4.13
Barley dust (BD) (*Hordeum vulgare*)	6.18	5.21	2.66	2.55	5.14	78.26	4.01
Bengal gram dust (BGD) (*Cicer arietinum*)	6.50	9.58	2.48	4.261	34.62	42.56	4.33
Soybean husk (SBH) (*Glycine max*)	5.49	6.64	3.80	5.42	31.25	47.40	3.33
Lentil husk (LH) (*Lens culinaris*)	6.09	11.47	2.25	6.76	19.93	53.50	4.36

* Results are mean of 4-5 determinations ± SEM.

** Dry matter basis.

Table 2. Ingredient composition of reference diet.

Ingredient (g/100 g as fed basis)	(%)
Casein (84% CP)	38.09
Gelatin (87.6% CP)	9.13
Dextrin	30.00
Corn oil	4.67
Codliver oil	2.33
Mineral mix[1]	4.00
Vitamin mix[1]	1.00
α-cellulose	8.78
Cr_2O_3	1.00
Carboxymethyl cellulose	1.00

[1] Halver, 1976.

Table 3. Proximate composition of reference and test diet (as fed basis)*.

Diet	Moisture**	Crude protein	Crude fat	Ash	Fibre	Carbohydrate	Cr_2O_3	Energy**
	(% dry matter)							kcal. g^{-1}
Reference diet (RD)	7.44	40.10	7.03	8.08	6.30	30.05	1.00	4.23
RB	6.96	31.58	5.43	8.23	7.65	39.46	0.69	3.98
RP	6.50	30.55	8.52	5.78	6.72	41.24	0.69	4.29
WB	6.58	31.12	6.05	6.20	7.21	42.12	0.72	4.13
RYC	6.32	29.80	5.99	4.62	5.39	47.18	0.70	4.27
CYC	6.20	29.88	5.09	4.72	5.36	48.05	0.70	4.22
RPS	6.47	29.90	4.98	5.11	5.14	47.70	0.70	4.20
CPS	6.30	29.90	4.98	5.18	5.06	47.88	0.70	4.21
BD	6.50	29.59	5.66	5.00	5.90	46.65	0.70	4.20
BGD	6.20	30.82	5.68	5.50	14.79	36.30	0.71	3.88
SBH	6.21	29.90	5.99	5.84	13.77	37.60	0.69	3.90
LH	6.20	31.40	5.53	6.26	10.39	39.52	0.70	4.00

* Results are mean triplicate runs ± SEM ($N = 3$).
** Dry matter basis.

Table - 4.
Proximate composition and Chromic Oxide (%), and gross energy content of faecal matter in *C. catla*, *L. rohita* and *C. mrigala* fed reference and test diets (dry weight basis).*

Ingredient	Dry Matter			Crude Protein			Fat			Ash			Fibre			Cr_2O_3			Carbohydrate			Energy (kcal g^{-1})		
	C#	R$	M@	C	R	M	C	R	M	C	R	M	C	R	M	C	R	M	C	R	M	C	R	M
RD	12.10 ±0.01	11.75 ±0.02	10.77 ±0.05	3.29 ±0.03	3.00 ±0.02	2.99 ±0.03	0.94 ±0.01	0.84 ±0.02	0.83 ±0.00	30.36 ±0.44	27.26 ±1.02	23.02 ±1.16	57.51 ±0.57	62.04 ±1.07	66.70 ±1.07	2.28 ±0.02	2.16 ±0.02	2.16 ±0.01	5.62 ±0.08	4.70 ±0.04	4.30 ±0.01	0.51 ±0.00	0.45 ±0.00	0.43 ±0.00
RB	17.40 ±0.04	16.31 ±0.02	18.27 ±0.03	5.73 ±0.03	5.41 ±0.05	4.98 ±0.03	0.79 ±0.01	0.89 ±0.07	0.82 ±0.08	23.20 ±1.06	24.62 ±0.81	24.77 ±0.71	49.35 ±0.95	50.60 ±0.79	51.88 ±0.73	2.23 ±0.04	2.16 ±0.02	2.11 ±0.01	18.70 ±0.03	16.32 ±0.02	15.44 ±0.03	1.17 ±0.03	1.08 ±0.00	1.01 ±0.00
RP	33.09 ±0.03	32.59 ±0.07	30.54 ±0.10	6.04 ±0.02	5.73 ±0.01	5.45 ±0.02	1.74 ±0.05	1.70 ±0.01	1.65 ±0.01	24.15 ±0.64	24.90 ±0.88	23.25 ±0.89	26.19 ±0.57	28.43 ±1.00	30.38 ±0.82	2.42 ±0.04	2.38 ±0.04	2.43 ±0.03	39.46 ±0.06	36.86 ±0.08	36.84 ±0.07	2.18 ±0.01	2.05 ±0.00	2.02 ±0.01
WB	19.36 ±0.06	18.70 ±0.03	17.05 ±0.02	3.94 ±0.03	3.35 ±0.01	3.22 ±0.04	1.20 ±0.02	0.90 ±0.02	0.89 ±0.04	28.09 ±1.08	26.41 ±0.62	25.92 ±0.61	47.23 ±0.72	51.30 ±0.56	53.45 ±0.58	1.96 ±0.01	1.74 ±0.04	1.66 ±0.02	17.58 ±0.04	16.30 ±0.06	14.86 ±0.06	1.05 ±0.03	0.97 ±0.00	0.90 ±0.00
RYC	35.13 ±0.06	33.15 ±0.05	34.12 ±0.03	7.09 ±0.04	6.95 ±0.04	6.60 ±0.04	1.81 ±0.05	1.80 ±0.03	1.31 ±0.06	21.88 ±0.89	23.59 ±0.83	20.93 ±0.76	31.95 ±1.07	28.81 ±0.92	32.80 ±0.99	2.42 ±0.02	2.45 ±0.02	2.30 ±0.04	34.85 ±0.07	36.40 ±0.05	36.06 ±0.08	2.05 ±0.01	2.11 ±0.00	2.06 ±0.01
CYC	16.17 ±0.11	16.09 ±0.06	15.30 ±0.06	3.22 ±0.04	3.07 ±0.04	2.94 ±0.04	0.85 ±0.05	0.86 ±0.07	0.72 ±0.05	22.00 ±0.78	26.07 ±0.76	25.43 ±0.68	55.50 ±0.86	51.89 ±0.93	53.50 ±0.81	2.06 ±0.03	2.00 ±0.02	1.85 ±0.01	16.37 ±0.02	16.13 ±0.08	15.56 ±0.05	0.96 ±0.01	0.94 ±0.01	0.89 ±0.01
RPS	38.05 ±0.03	38.96 ±0.09	36.29 ±0.03	7.91 ±0.01	6.92 ±0.05	6.37 ±0.02	1.70 ±0.04	2.04 ±0.04	2.07 ±0.04	23.27 ±0.83	20.63 ±0.83	19.74 ±0.94	25.59 ±0.88	29.14 ±0.92	30.50 ±0.82	2.44 ±0.03	2.42 ±0.01	2.52 ±0.02	39.09 ±0.06	38.85 ±0.08	38.80 ±0.13	2.27 ±0.01	2.17 ±0.05	2.20 ±0.01
CPS	15.51 ±0.02	15.32 ±0.02	14.99 ±0.01	3.05 ±0.06	3.04 ±0.07	2.72 ±0.02	0.99 ±0.01	1.00 ±0.01	0.84 ±0.02	20.21 ±0.76	19.64 ±0.78	18.92 ±0.67	53.66 ±0.60	54.50 ±0.81	56.93 ±0.65	2.19 ±0.02	2.12 ±0.01	2.03 ±0.02	19.90 ±0.08	19.70 ±0.04	18.56 ±0.06	1.09 ±0.01	1.08 ±0.02	1.01 ±0.01
BD	33.95 ±0.04	35.74 ±0.01	36.71 ±0.02	8.08 ±0.05	7.85 ±0.05	6.97 ±0.04	2.89 ±0.07	2.07 ±0.04	2.55 ±0.05	21.03 ±0.91	20.54 ±1.61	18.94 ±1.12	39.63 ±0.98	43.93 ±1.74	47.00 ±1.07	2.64 ±0.01	2.50 ±0.01	2.42 ±0.01	25.75 ±0.08	23.11 ±0.05	23.12 ±0.06	1.82 ±0.00	1.62 ±0.01	1.57 ±0.00
BGD	21.99 ±0.01	20.09 ±0.14	19.95 ±0.04	6.72 ±0.02	6.08 ±0.04	5.93 ±0.06	1.93 ±0.02	1.80 ±0.03	1.71 ±0.05	25.50 ±0.69	21.80 ±0.60	19.67 ±0.58	41.80 ±0.77	48.83 ±0.63	52.28 ±0.70	1.99 ±0.02	1.74 ±0.03	1.61 ±0.00	22.06 ±0.05	19.75 ±0.02	18.80 ±0.11	1.50 ±0.01	1.35 ±0.00	1.30 ±0.00
SBH	20.09 ±0.07	19.86 ±0.06	18.39 ±0.05	5.60 ±0.04	5.07 ±0.04	4.93 ±0.06	1.73 ±0.04	1.61 ±0.01	1.51 ±0.01	23.56 ±0.68	20.18 ±0.68	19.48 ±0.65	48.46 ±0.06	51.53 ±0.75	55.43 ±0.72	1.95 ±0.03	1.81 ±0.01	1.74 ±0.03	18.70 ±0.04	19.80 ±0.06	16.91 ±0.06	1.27 ±0.01	1.28 ±0.00	1.14 ±0.00
LH	20.09 ±0.01	19.59 ±0.36	19.76 ±0.06	6.36 ±0.02	5.95 ±0.04	5.40 ±0.04	1.81 ±0.02	1.73 ±0.04	1.73 ±0.07	21.58 ±0.65	20.10 ±0.74	19.26 ±0.71	48.44 ±0.73	52.80 ±0.93	56.03 ±0.86	1.91 ±0.01	1.92 ±0.01	1.88 ±0.01	19.90 ±0.05	17.50 ±0.12	15.70 ±0.04	1.38 ±0.01	1.24 ±0.01	1.14 ±0.01

*Results are mean of triplicate runs ±SEM (N = 3)
#*C. catla*; $*L. rohita*; @*C. mrigala*.

Table - 5.
Apparent dry matter and nutrient digestibility (%) of reference and test diets in Indian major carps.*

	Dry Matter			Crude Protein			Fat			Carbohydrate			Energy		
Ingre-dient	C#	R$	M@	C	R	M	C	R	M	C	R	M	C	R	M
RD	94.30 ±0.11	94.14 ±0.01	94.63 ±0.10	96.42 ±0.04	96.55 ±0.03	96.57 ±0.09	94.19 ±0.17	94.52 ±0.12	94.58 ±0.10	91.84 ±0.01	92.79 ±0.05	93.39 ±0.11	94.69 ±0.06	95.10 ±0.06	95.30 ±0.10
RB	94.25 ±0.11	94.44 ±0.03	93.63 ±0.01	94.43 ±0.05	94.57 ±0.02	94.88 ±0.04	95.76 ±0.16	94.79 ±0.45	95.12 ±0.49	85.43 ±0.22	87.19 ±0.29	87.30 ±0.01	90.80 ±0.12	91.41 ±0.05	91.73 ±0.04
RD	89.98 ±0.21	89.96 ±0.24	90.79 ±0.18	94.40 ±0.14	94.60 ±0.13	94.97 ±0.10	94.23 ±0.27	94.25 ±0.07	94.53 ±0.11	72.90 ±0.69	74.27 ±0.56	74.81 ±0.50	85.62 ±0.38	86.26 ±0.29	86.68 ±0.25
WB	92.44 ±0.07	91.75 ±0.22	92.02 ±0.16	95.39 ±0.10	95.56 ±0.14	95.53 ±0.01	92.77 ±0.01	93.85 ±0.34	93.65 ±0.29	84.76 ±0.13	84.04 ±0.35	84.75 ±0.24	90.44 ±0.10	90.34 ±0.28	90.62 ±0.13
RYC	89.21 ±0.11	89.94 ±0.13	88.99 ±0.25	93.15 ±0.06	93.37 ±0.06	93.30 ±0.17	92.16 ±0.43	91.45 ±0.22	93.40 ±0.17	78.75 ±0.19	78.06 ±0.27	76.90 ±0.46	86.17 ±0.10	85.95 ±0.19	85.42 ±0.40
CYC	94.17 ±0.02	94.03 ±0.05	93.87 ±0.03	96.36 ±0.08	96.42 ±0.06	96.30 ±0.04	94.39 ±0.37	94.12 ±0.46	94.73 ±0.35	88.48 ±0.14	88.31 ±0.11	87.84 ±0.04	92.34 ±0.14	92.25 ±0.13	92.05 ±0.07
RPS	88.30 ±0.27	87.96 ±0.14	89.20 ±0.18	92.40 ±0.21	93.31 ±0.16	94.07 ±0.19	90.17 ±0.42	88.19 ±0.14	88.46 ±0.08	76.43 ±0.58	76.44 ±0.36	77.36 ±0.34	84.47 ±0.42	84.63 ±0.24	85.40 ±0.22
CPS	94.69 ±0.08	94.59 ±0.08	94.47 ±0.10	96.73 ±0.01	96.64 ±0.13	96.86 ±0.13	93.63 ±0.02	93.38 ±0.14	94.20 ±0.36	86.69 ±0.16	86.37 ±0.19	86.60 ±0.23	91.69 ±0.18	91.88 ±0.02	91.69 ±0.22
BD	89.94 ±0.35	89.35 ±0.11	88.70 ±0.11	92.82 ±0.03	92.62 ±0.16	93.22 ±0.02	86.57 ±0.24	89.80 ±0.33	87.06 ±0.04	84.97 ±0.41	86.20 ±0.17	86.37 ±0.18	88.56 ±0.01	89.24 ±0.17	89.20 ±0.08
BGD	91.68 ±0.02	91.36 ±0.09	90.64 ±0.03	92.26 ±0.06	92.01 ±0.15	90.91 ±0.53	87.96 ±0.14	87.16 ±0.12	86.77 ±0.49	78.40 ±0.08	77.99 ±0.26	77.25 ±0.03	86.27 ±0.21	85.88 ±0.28	85.33 ±0.11
SBH	92.40 ±0.02	91.93 ±0.02	92.22 ±0.07	93.36 ±0.10	93.54 ±0.01	93.47 ±0.13	89.75 ±0.33	90.26 ±0.26	90.01 ±0.04	82.36 ±0.09	79.93 ±0.14	82.17 ±0.11	88.41 ±0.22	87.50 ±0.04	88.41 ±0.20
LH	92.13 ±0.04	92.39 ±0.12	92.14 ±0.01	92.56 ±0.01	93.09 ±0.04	93.59 ±0.02	87.96 ±0.17	88.59 ±0.29	88.34 ±0.53	81.48 ±0.07	83.87 ±0.04	85.18 ±0.06	87.36 ±0.04	88.67 ±0.11	89.89 ±0.36

*Results are mean of triplicate runs ±SEM (N = 3)
#*C. catla*; $*L. rohita*; @*C. mrigala*.

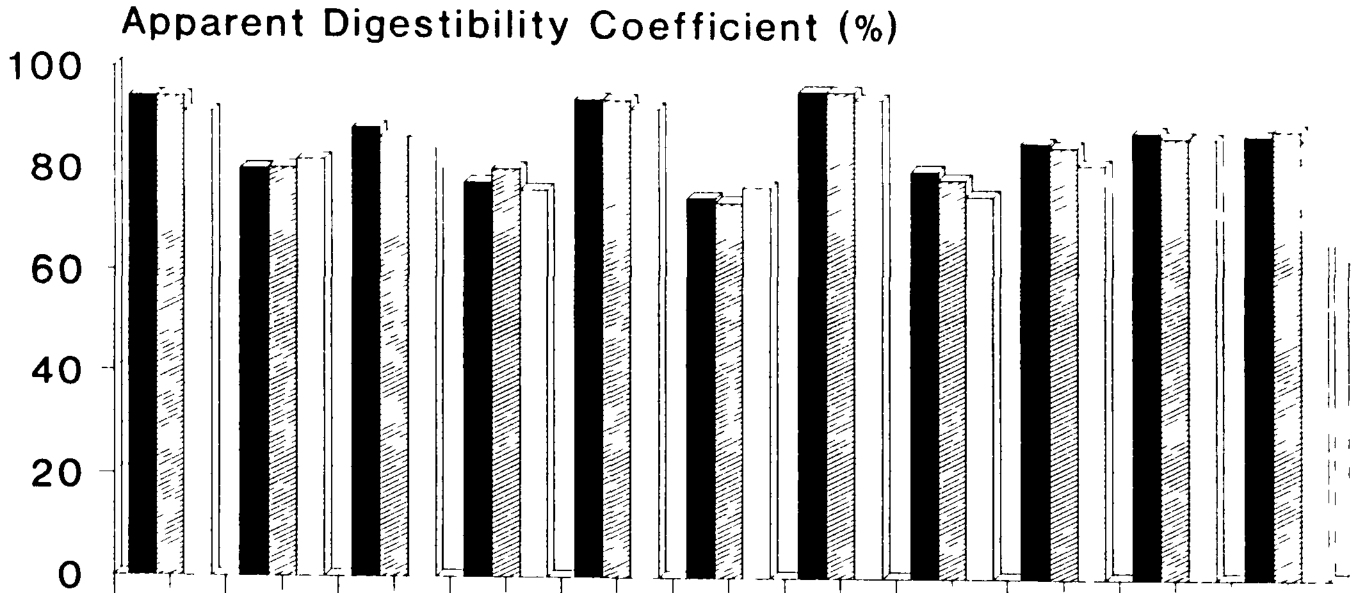
Table - 6.

Apparent digestibility coefficients (%) of dry matter and nutrients of test ingredients in Indian major carps.*

Ingredient	Dry Matter			Crude Protein			Fat			Carbohydrate			Energy		
	C#	R$	M@	C	R	M	C	R	M	C	R	M	C	R	M
RB	94.15^{f} ±0.51	94.24^{g} ±0.72	91.28^{e} ±0.20	89.77^{d} ±0.26	89.93^{e} ±0.01	90.95^{d} ±0.09	98.74^{h} ±0.45	95.43^{f} ±1.78	93.68^{de} ±1.88	70.47^{f} ±0.76	74.12^{g} ±1.10	73.07^{f} ±0.21	81.71^{f} ±0.52	82.80^{g} ±0.30	83.40^{g} ±0.37
RP	79.90^{c} ±0.44	80.21^{c} ±0.77	81.83^{c} ±0.36	89.67^{d} ±0.37	90.04^{e} ±0.37	91.23^{d} ±0.11	94.31^{f} ±0.51	93.63^{ef} ±0.04	94.40^{de} ±0.13	28.69^{a} ±2.26	31.01^{a} ±1.73	31.46^{a} ±1.42	64.44^{b} ±1.60	65.62^{b} ±0.82	66.58^{b} ±0.61
WB	88.12^{e} ±0.70	86.16^{e} ±0.75	86.25^{d} ±0.06	92.97^{e} ±0.40	93.25^{f} ±0.54	93.12^{e} ±0.23	89.44^{e} ±0.38	92.30^{e} ±1.40	91.47^{d} ±0.72	68.24^{f} ±0.46	63.62^{e} ±1.29	64.59^{c} ±0.78	80.51^{f} ±0.47	79.22^{f} ±1.06	79.70^{f} ±0.19
RYC	77.33^{b} ±0.11	80.14^{c} ±0.42	75.83^{a} ±0.59	85.52^{b} ±0.11	85.95^{c} ±0.28	85.69^{b} ±0.19	85.98^{d} ±0.02	84.32^{d} ±0.46	90.63^{d} ±0.78	48.19^{c} ±0.62	43.69^{c} ±0.78	38.41^{b} ±1.30	66.29^{c} ±0.20	65.27^{b} ±0.98	62.38^{a} ±1.10
CYC	93.88^{f} ±0.33	93.76^{g} ±0.19	92.10^{e} ±0.14	96.20^{e} ±0.35	96.12^{g} ±0.25	95.68^{f} ±0.35	94.84^{fg} ±1.63	93.18^{e} ±1.82	95.08^{de} ±1.39	80.64^{h} ±0.50	77.87^{h} ±0.50	74.89^{f} ±0.37	86.84^{h} ±0.62	85.60^{gh} ±0.56	84.46^{g} ±0.49
RPS	74.31^{a} ±1.15	73.52^{a} ±0.52	76.53^{ab} ±0.82	83.00^{a} ±0.78	85.75^{c} ±0.59	85.25^{bc} ±0.65	80.79^{c} ±1.79	73.43^{b} ±0.19	74.18^{b} ±0.05	40.46^{b} ±1.98	38.29^{b} ±1.31	39.94^{b} ±1.36	60.61^{a} ±1.53	60.19^{a} ±0.92	62.31^{a} ±0.96
CPS	95.60^{fg} ±0.53	95.64^{h} ±0.29	94.10^{f} ±0.56	97.46^{g} ±0.06	96.83^{g} ±0.50	97.55^{g} ±0.51	92.31^{f} ±0.46	90.72^{e} ±0.73	93.30^{d} ±1.06	74.66^{g} ±0.56	71.39^{f} ±0.77	70.76^{f} ±1.00	84.69^{g} ±0.72	84.35^{g} ±0.07	83.62^{g} ±0.98
B D	79.78^{c} ±1.17	78.18^{b} ±0.39	74.86^{a} ±0.61	84.41^{ab} ±0.19	83.43^{b} ±0.43	85.42^{b} ±0.28	68.79^{a} ±0.40	78.79^{c} ±1.37	69.50^{a} ±0.10	68.93^{f} ±1.39	70.84^{f} ±0.69	69.97^{e} ±0.85	74.24^{e} ±0.14	75.55^{e} ±0.69	74.98^{d} ±0.52
BGD	85.56^{d} ±0.34	84.88^{d} ±0.24	81.32^{c} ±0.12	82.56^{a} ±0.29	81.40^{a} ±0.57	77.70^{a} ±1.96	73.43^{b} ±0.87	70.00^{a} ±0.18	68.55^{a} ±1.40	47.04^{c} ±0.32	43.47^{c} ±1.00	39.59^{b} ±0.34	66.63^{c} ±0.82	64.37^{b} ±1.07	62.08^{a} ±0.59
SBH	87.78^{e} ±0.25	86.76^{e} ±0.05	86.60^{d} ±0.47	86.22^{bc} ±0.41	86.52^{cd} ±0.16	86.23^{b} ±0.64	79.39^{c} ±2.13	80.32^{c} ±1.13	79.28^{c} ±0.44	60.23^{e} ±0.47	49.92^{d} ±0.34	55.98^{c} ±0.61	73.74^{e} ±0.88	69.75^{c} ±0.26	72.35^{c} ±0.90
LH	87.08^{e} ±0.11	88.31^{f} ±0.43	86.32^{d} ±0.19	83.56^{a} ±0.06	85.02^{c} ±0.26	86.63^{b} ±0.29	73.42^{b} ±1.36	74.75^{b} ±1.23	73.78^{b} ±2.83	57.31^{d} ±0.29	63.05^{e} ±0.25	66.01^{d} ±0.05	70.24^{d} ±0.37	73.65^{d} ±0.49	77.27^{e} ±0.60

***Results are mean of triplicate runs ±SEM (N = 3). Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.**
#*C. catla;* $*L. rohita;* @*C. mrigala.*

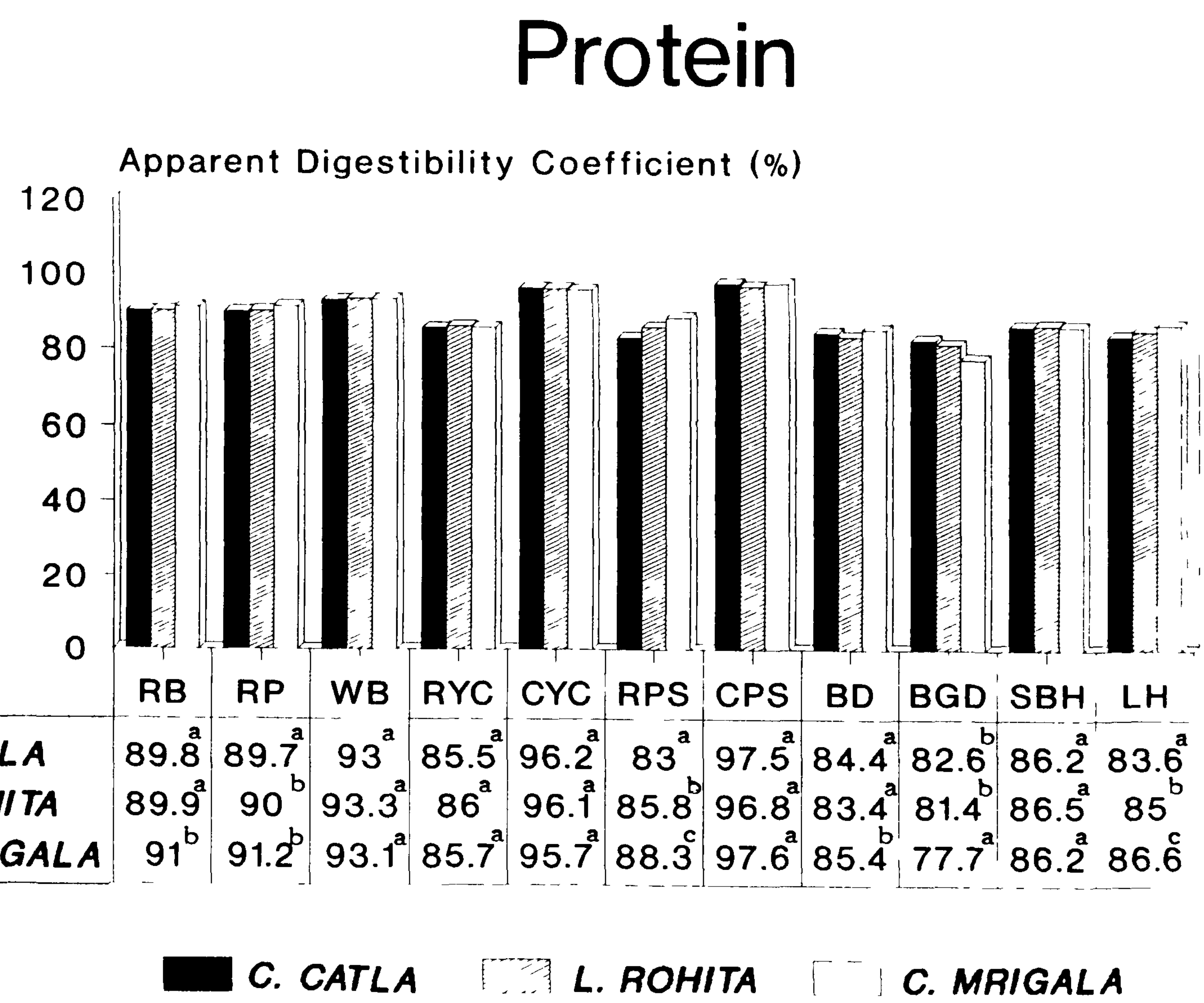
Dry matter



	RB	RP	WB	RYC	CYC	RPS	CPS	BD	BGD	SBH	LH
C. CATLA	94.2^{b}	79.9^{a}	88.1^{b}	77.3^{b}	93.9^{b}	74.3^{a}	95.6^{b}	79.8^{b}	85.6^{b}	87.8^{b}	87.1^{a}
L. ROHITA	94.2^{b}	80.2^{a}	86.2^{a}	80.1^{c}	93.8^{b}	73.5^{a}	95.6^{b}	78.2^{b}	84.9^{b}	86.8^{a}	88.3^{b}
C. MRIGALA	91.3^{a}	81.8^{b}	86.3^{a}	75.8^{a}	92.1^{a}	76.5^{a}	94.1^{a}	74.9^{a}	81.3^{a}	86.6^{a}	86.3^{a}

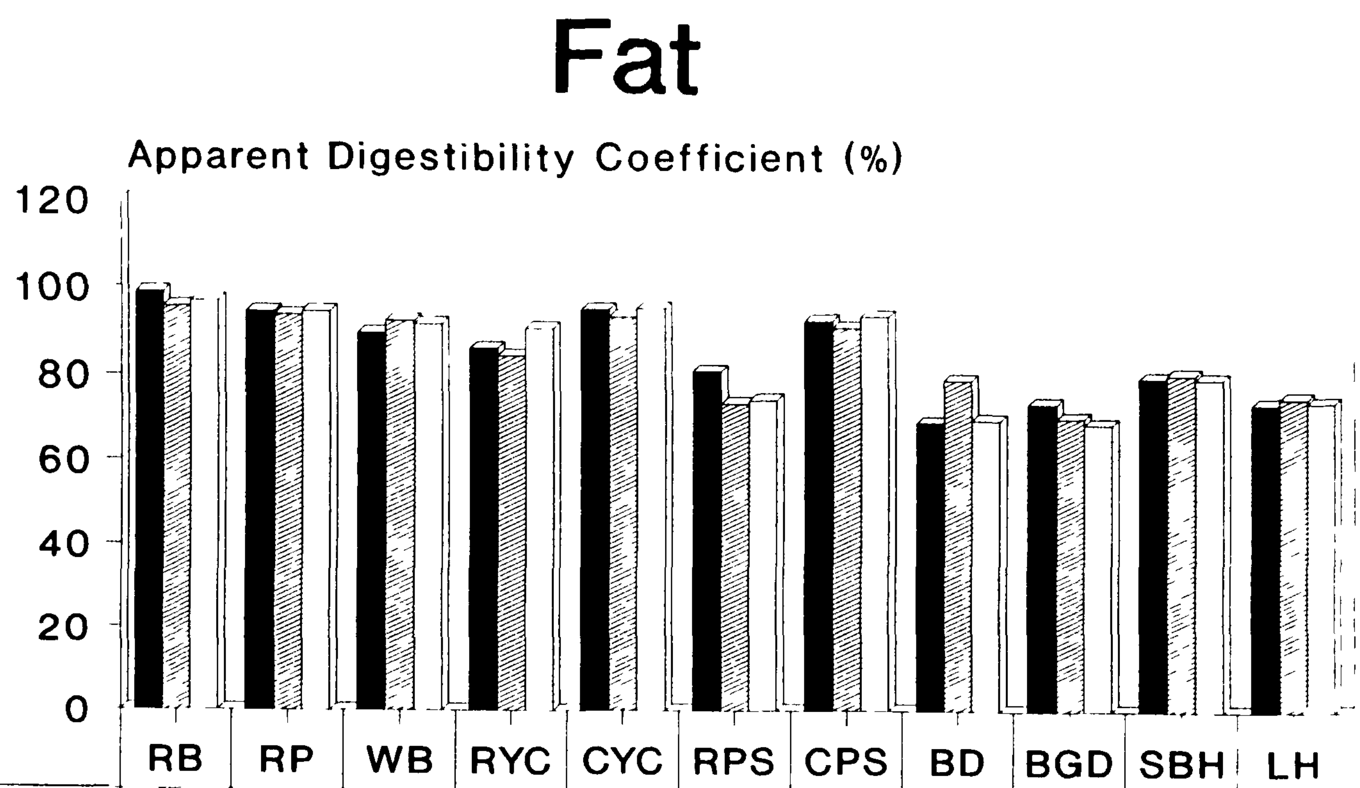
C. CATLA *L. ROHITA* *C. MRIGALA*

Fig. 1. Comparison of apparent dry matter digestibility coefficient in three fish species fed the similar test ingredient. Values in the same column not sharing the same superscripts are significantly ($P<0.05$) different.



	RB	RP	WB	RYC	CYC	RPS	CPS	BD	BGD	SBH	LH
C. CATLA	89.8[a]	89.7[a]	93[a]	85.5[a]	96.2[a]	83[a]	97.5[a]	84.4[a]	82.6[b]	86.2[a]	83.6[a]
L. ROHITA	89.9[a]	90[b]	93.3[a]	86[a]	96.1[a]	85.8[b]	96.8[a]	83.4[a]	81.4[b]	86.5[a]	85[b]
C. MRIGALA	91[b]	91.2[b]	93.1[a]	85.7[a]	95.7[a]	88.3[c]	97.6[a]	85.4[b]	77.7[a]	86.2[a]	86.6[c]

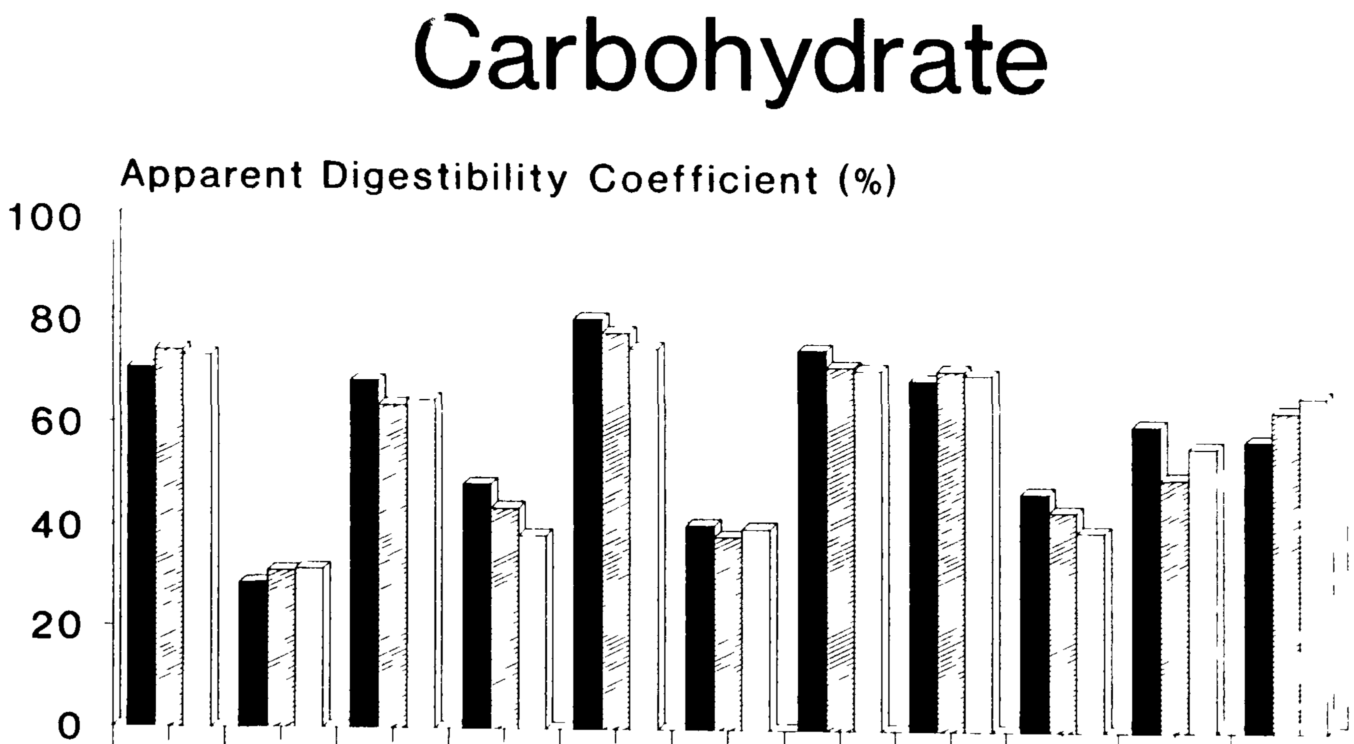
Fig. 2. Comparison of apparent protein digestibility coefficient in three fish species fed the similar test ingredient. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.



	RB	RP	WB	RYC	CYC	RPS	CPS	BD	BGD	SBH	LH
C. CATLA	98.7[a]	94.3[a]	89.4[a]	86[b]	94.8[a]	80.8[b]	92.3[a]	68.8[a]	73.4[b]	79.4[a]	73.4[a]
L. ROHITA	95.4[a]	93.6[a]	92.3[a]	84.3[a]	93.2[a]	73.4[a]	90.7[a]	78.8[b]	70[a]	80.3[a]	74.8[a]
C. MRIGALA	96.7[a]	94.4[a]	91.5[a]	90.6[c]	95.1[a]	74.2[a]	93.3[a]	69.5[c]	68.5[a]	79.3[a]	73.8[a]

C. CATLA *L. ROHITA* *C. MRIGALA*

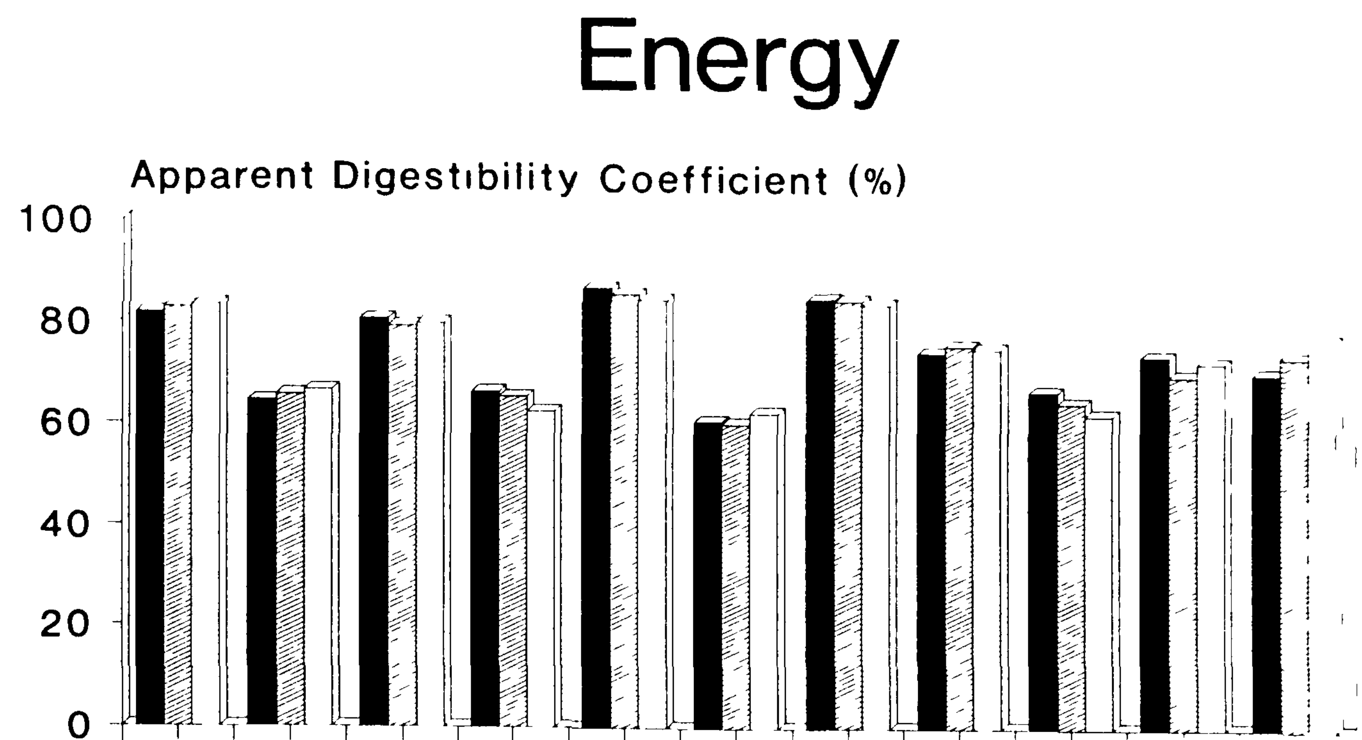
Fig. 3. Comparison of fat digestibility coefficient in three fish species fed the similar test ingredient. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.



	RB	RP	WB	RYC	CYC	RPS	CPS	BD	BGD	SBH	LH
C. CATLA	70.5^{a}	28.7^{a}	68.2^{b}	48.2^{c}	80.6^{c}	40.5^{a}	74.7^{b}	68.9^{a}	47^{c}	60.3^{c}	57.3^{a}
L. ROHITA	74.1^{b}	31.1^{a}	63.6^{a}	43.7^{b}	77.9^{b}	38.3^{a}	71.4^{a}	70.8^{a}	43.5^{b}	49.9^{a}	63.1^{b}
C. MRIGALA	73.1^{b}	31.5^{a}	64.6^{a}	38.4^{a}	74.9^{a}	39.9^{a}	70.8^{a}	70^{a}	39.6^{a}	56^{b}	66^{c}

C. CATLA *L. ROHITA* *C. MRIGALA*

Fig. 4. Comparison of apparent carbohydrate digestibility coefficient in three fish species fed the similar test ingredient. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.



	RB	RP	WB	RYC	CYC	RPS	CPS	BD	BGD	SBH	LH
C. CATLA	81.7^{a}	64.4^{a}	80.5^{a}	66.3^{b}	86.8^{b}	60.6^{a}	84.7^{a}	74.2^{a}	66.6^{b}	73.7^{b}	70.2^{a}
L. ROHITA	82.8^{ab}	65.6^{a}	79.2^{a}	65.3^{b}	85.6^{ab}	60.2^{a}	84.4^{a}	75.6^{a}	64.4^{ab}	69.8^{a}	73.7^{b}
C. MRIGALA	83.4^{b}	66.6^{a}	79.7^{a}	62.4^{a}	84.5^{a}	62.3^{a}	83.6^{a}	75^{a}	62.1^{a}	72.4^{b}	77.3^{c}

C. CATLA *L. ROHITA* *C. MRIGALA*

Fig. 5. Comparison of apparent energy digestibility coefficient in three fish species fed the similar test ingredient. Values in the same column not sharing the same superscripts are significantly ($P < 0.05$) different.

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APPENDIX

Appendix

I. Source of synthetic/artificial ingredients used.

1. Protein

Vitamin free casein (84% CP)	- ICN pharmaceuticals, Inc. Life Sciences Grade Cleaveland, Ohio (USA).
Gelatin (87.6% CP)	- Loba-Chemie Indoaustranal Co.,Bombay (India).
Fish meal	- Laboratory prepared
Soybean meal	- Expeller grade

2. Carbohydrates

D-Glucose ($C_6 H_{12} O_6$=180.16)	- Loba-Chemie Indoaustranal Co.,Bombay (India).
Fructose ($C_6 H_{12} O_6$=180.16)	- ---do---
Maltose ($C_6H_{12} O_{11}.H_2O$=360.32)	- ---do---
Sucrose ($C_{12}H_{22}O_{11}$=342.30)	- ---do---
Dextrin ($((C_6H_{10}O_5)_n.X H_2O)$	- ---do---
Corn starch	- Commercial grade
Bread flour	- ---do---

3. Oil

Corn oil (Cornola)	- Commercial grade
Cod liver oil (Seven seas)	- Universal Generics Pvt. Ltd.,Thane (India).

4. Vitamins & Minerals	- British Drug House,Ltd., Poole (England) - Loba-Chemie Indoaustranal Co.,Bombay (India).

5. Fibre

α-cellulose	- Sigma Chemicals Co., St. Louis,MO.(USA)

6. Binder

Carboxymethyl cellulose	- Loba-Chemie Indoaustranal Co.,Bombay (India)

7. All other artificial ingredients were of commercial grade.

II.Chemicals used.

Potassium permanganate ($KMnO_4$=158.04)	- Qualigens Fine Chemicals,Bombay (India)
Tricane methanesulfonate (MS 222)	- Ayerst Laboratories Inc.,New York (USA)
Petroleum Spirit (40 - 6 0°C) (Petroleum ether)	- Qualigens Fine Chemicals,Bombay (India).
Sulphuric Acid (H_2SO_4=98.08)	- E.Merck (India) Ltd., Bombay. AR grade.
Ammonium Sulphate ($(NH_4)_2SO_4$=132.14)	- Qualigens Fine Chemicals,Bombay (India).
Potassium persulphate ($K_2S_2O_8$=270.33)	- Loba-Chemie Indoaustranal Co.,Bombay (India).
Mercuric Iodide (HgI_2=454.40)	- Qualigens Fine Chemicals,Bombay (India).
Potassium Iodide(KI=166.01)	- E.Merck (India) Ltd., Bombay.
Sodium Hydroxide(NaOH=40.00)	- Loba-Chemie Indoaustranal Co.,Bombay (India).
Benzoic acid ($C_7H_6O_6$=122.12)	- E.Merck (India) Ltd., Bomaby.
Octyl alcohol ($C_8H_{10}O$=130.23) (Capryl alcohol)	- Sigma Chemical Co., St.Louis,MO (USA).
Hydrochloric acid (HCl about 36%)sp.gr.1.18	- E.Merck (India)Ltd., Bombay.AR grade.
Nitric acid (HNO_3=63.01)	- Qualigens Fine Chemicals,Bombay.

Perchloric acid 60% ($HClO_4$=100.46)	-	---do---
Chromic oxide (Cr_2O_3=151.99)	-	Ajax Chemicals, Sydney (Australia).
Heparin Sodium Injection (I.P.)	-	Biological Evans Ltd., Hyderabad (India).
Trichloroacetic acid ($CCl_3.COOH$=163.4)	-	Glaxo laboratories (India) Ltd., Bombay.
O-toluidine (C_7H_9N) (2-aminotoluene)	-	E.Merck (India) Ltd., Bombay. GR grade.
Acetone ($(CH_3)_2CO$=58.08)	-	Qualigens Fine Chemicals, Bombay. LR grade.
Methanol (CH_3OH=32.04)	-	E.Merck (India) Ltd., Bombay.
Deionized H_2O	-	Laboratory prepared.

III. Glasswares used.

Corning^R and Borosil^R make glasswares were used.

IV. Statistical formulae used.

1. Mean $\bar{X} = \frac{\Sigma x_i}{N}$

where, x_i = weight of the ith fish

N = total number of fish

2. Stadard deviation (S.D.)

$$\sigma = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_i - \bar{x})^2}$$

3. Standard error of the mean (SEM)

$$SEM = \frac{\sigma}{\sqrt{n-1}}$$

where, σ = standard deviation

n = population number

4. Correlation (r)

The correlation coefficient between X and Y is

$$r = \frac{n \Sigma XY - \Sigma X \Sigma y}{\sqrt{[n \Sigma X^2 - (\Sigma X)^2][n \Sigma y^2 - (\Sigma y)^2]}}$$

Significance of (r) $t_{0.05} = \frac{r\sqrt{n-2}}{1-r^2}$

5. Linear regresssion y = a+bX

Line of regression of X on Y is

$(X-\overline{X}) = b_{XY}\ (Y-\overline{Y})$

where, $\overline{X}$ and $\overline{Y}$ are the means of X and Y values respectively, and

$$b_{XY} = \frac{n \Sigma XY - \Sigma X \Sigma y}{n \Sigma y^2 - (\Sigma Y)^2}$$

6. Second degree polynomial regression $y = a+bX + CX^2$

Normal equations :

$$\Sigma y = na + b\,\Sigma X + C\,\Sigma X^2 \quad ---(1)$$

$$\Sigma XY = a\Sigma X + b\,\Sigma X^2 + C\,\Sigma X^3 \quad ---(2)$$

$$\Sigma X^2 Y = a\Sigma X^2 + b\,\Sigma X^3 + C\,\Sigma X^4 \quad ---(3)$$

7. Oneway analysis of variance (ANOVA)

Set up of ANOVA table

Source of varition S.V.	Sum of squares S.S.	Degrees of freedom d.f.	Mean sum of squares $= \frac{S.S.}{d.f.}$	Variance ratio F
(i)Between samples	$\Sigma \frac{X^2}{n} - \frac{1}{N}(\Sigma X)^2$	$K-1=n_2$	V_2	$F=\frac{V_2}{V_1}$
(ii)Within samples	$\Sigma X^2 - \Sigma \frac{X^2}{n}$	$N-K=n_1$	V_1	
Total	$\Sigma X^2 - \frac{1}{N}(\Sigma X)^2$	$N-1$		

where, X = Sample total, n = sample size

K = Number of samples, N = Total number of observations

ΣX= Grand total of all sample observations

and ΣX^2=Grand total of squares of all samples observations

If $F > F_{0.05}(n_2, n_1)$ the samples are significantly different.

----------x----------